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THE

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UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE

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RAPID ANALYSIS OF THE AMINO ACID CONTENT OF FISH DIETS

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Western Fish Nutrition Laboratory, Cook, Washington

KNOWLEDGE OF THE SPECIFIC AMINO ACID CONTENT OF DIETARY PROTEIN is a most important prerequisite in the formulation of balanced rations. Growth of fish, as with other animals, is directly dependent on the intake and balance of the 10 indispensable amino acids contained in the protein component of the diet (Halver, DeLong, and Mertz, 1957, 1959; Harper 1958). Although extreme variation in hatchery diets is known to exist (Wood, Yasutake, Woodall, and Halver, 1957), hatchery managers know that protein generally constitutes the bulk of the diet and is the most expensive component to supply. Recent work indicates a direct relationship between environmental temperature and quantitative protein requirement over a range of 40 to 55 percent protein for maximum growth (DeLong, Halver, and Mertz, 1958). This further complicates the protein picture and emphasizes that future improvements in fish diets will stem from integration of economically available amino acid sources.

The fish nutritionist is confronted with the problem of testing protein for relative nutritional value. With other animals, the three most frequently employed methods are: Biological Value determination, feeding trials to determine Protein Efficiency Ratio, and amino acid analysis to provide Chemical Score.

Amino acid analysis can be applied to the problem of determining protein quality by revealing the amount of each indispensable amino acid present. Maynard and Loosli (1956) and Block and Mitchell (1946-47) cite the good correlation between Biological Value and the indispensable amino acid spectrum of the protein. Such information has found practical application in other areas of animal husbandry by providing a basis for mixing complementary amino acid sources into a balanced ration (Maynard and Loosli 1956). However, it must be recognized that Chemical Score does not reflect digestibility of the protein or availability of the constituent amino acids; biological methods are still necessary to tell the complete amino acid story.

Materials and Methods

Two commercially available diets and casein, a classical reference protein, were assayed. The diets and casein were first extracted three times with ether to remove excess lipid. The samples were then dried, and total protein was measured by standard AOAC microkjeldahl methods (Horwitz 1955). Hydrolysis of the sample was performed with a 20-fold excess of 5.7N HCl in sealed evacuated Pyrex tubes for 7.5 hours under 15 pounds' pressure. The excess HCl was removed by vacuum distillation at 50° C. Samples were adjusted to pH 4.0 and filtered through sintered glass filters to remove insoluble humin (Henderson and Snell 1948). The hydrolysates were diluted to volume and aliquots were removed for the various assay procedures.

The hydrolysis for tryptophan assay was performed under alkaline conditions. Thus

10.0 ml. of 5.0N NaOH per 0.5-gm. samples was placed in sealed evacuated Pyrex tubes and autoclaved for 7.5 hours at 15 pounds' pressure (Henderson and Snell 1948). All hydrolysates were adjusted to pH 6.8 and diluted to fall within the assay range of the organism (Steele, Sauberlick, Reynolds, and Baumann, 1949). All standards used in this study were prepared from vacuum-dried, chromatographically pure amino acids.

The microbiological assays were performed in accordance with the procedure of Steele et al. (1949). The medium is similar for all assays, varying only with respect to the amino acids deleted; also, a single strain of organism, *Leuconostoc mesenteroides* P-60, can be used for all assays.

The enzymatic analyses were generally as described by Gale (1945) and Meister, Sober, and Tice (1951a, 1951b). This is a simple, convenient manometric technique employing commercially available enzymes. The decarboxylases discussed in this report are limited to *l*-lysine, *l*-arginine, and *l*-histidine decarboxylases.[1] The enzyme attacks a specific amino acid as follows (Gale 1947):

$$R\text{-}CHNH_2\text{-}COOH \xrightarrow{\text{Enzyme}} R\text{-}CH_2NH_2 + CO_2.$$

The CO_2 released was measured in a conventional Warburg apparatus and was proportional to the amount of amino acid present in the substrate. Acetate buffer, 0.5M, was used at three different pH levels: pH 4.5 for the *l*-histidine decarboxylase; pH 5.2 for *l*-arginine decarboxylase; and pH 6.0 for *l*-lysine decarboxylase. Aliquots of the hydrolysates were adjusted to desired pH. Flask constants were calculated on the basis of 3.0 ml. total contents (Umbreit, Burris, and Stauffer, 1957). The assays were carried out at 37° C. in a conventional circular Warburg. One flask and manometer containing 3.0 ml. buffer, and duplicate standards containing 1.0 ml. of an amino acid (0.0075M) and 1.0 ml. of buffer, were prepared. To the remaining flasks, 1.0 ml. of the unknown plus 1.0 ml. of buffer were added. A 1.0-ml. aqueous suspension of the enzyme containing 10 mg. of the dried cell suspension (for the histidine assay, 25 mg. were employed) was added to the sidearm of each flask. The flasks were sealed to the manometers, placed in the water bath, and agitated for 20 minutes. The manometers were then adjusted to zero and closed. A corrected zero reading was obtained after an additional 5 to 10 minutes' agitation. The flasks were tipped, and the contents of the sidearm mixed with the buffer and substrate. The flasks and manometers were replaced in the water bath and agitated, and the reaction was allowed to proceed until no further CO_2 was evolved (usually about 20 minutes). The CO_2 evolved was measured in microliters, and the calculation to convert microliters of CO_2 to milligrams of amino acid was as follows:

$$\frac{\mu l}{224} \times 1.462 = \text{mg. } l\text{-lysine.}$$

The third method of analysis to be discussed in this report is paper chromatography, a technique which in recent years has developed into one of the most versatile tools in the laboratory. Strain (1952) defined chromatography as follows: "Chromatography is an analytical technique for resolution of solutes, in which separation is made by differential migration in porous medium, and migration is caused by flow of solvent."

Because partition coefficients of amino acids vary with each solvent system, it is possible to select various solvents to resolve specific amino acids. McFarren (1951) described a series of buffered phenols and alcohols capable of resolving 18 amino acids. Roland and Gross (1954) employed 2 solvent systems which will resolve 16 amino acids sufficiently well for quantitation. Block, Durrum, and Zweig (1958) employed a series of solvents and types of papers to resolve 18 amino acids. None of the methods listed above successfully separated threonine, valine, methionine, leucine, isoleucine, and phenylalanine on a single chromatogram. G. R. Dixon described (in a personal communication, 1956) a solvent system which resolved these amino acids, as well as alanine, tyrosine, proline, and cystine, on a single uni-dimensional paper chromatogram (figure 1).

The procedure of the assays using this solvent (Dixon, personal communication, 1956) was as follows: Whatman No. 3MM paper was cut into strips 9 inches by 22 inches, dipped in the buffer (see below), and allowed to dry thoroughly. To a narrow

[1] The other decarboxylating enzymes presently available attack *l*-tyrosine, *l*-aspartic acid, *l*-glutamic acid, and *l*-ornithine, and may be obtained from Worthington Biochemical Corporation, Freehold, New Jersey.

edge of this paper, a folded absorbent pad of Whatman No. 1 paper was stapled. A base line was drawn 4.5 inches from the opposite edge of the buffer-impregnated paper and dotted at intervals of 2.0 cm. The material to be chromatographed was prepared according to Block, Durrum, and Zweig (1958) and applied along the base line with micropipettes in aliquots no larger than 5 µl. A draft of warm air from a common hair dryer reduced the drying time and aided in maintaining a small spot. The paper was then folded and suspended as indicated (figure 2) in a chamber constructed of plywood and measuring 9.5 by 14.5 by 36 inches. Wooden cleats fastened to the narrow sides of the

(Right)

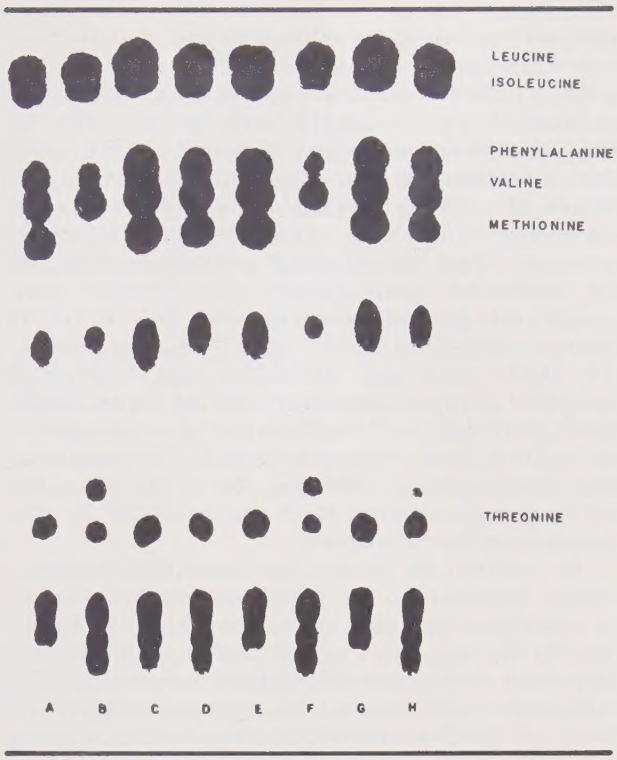

FIGURE 1.-- Resolution of six indispensable amino acids. Paper chromatogram showing resolution of six essential amino acids. A, E, and G are crystalline amino acid standards; B and F designate aliquots of hydrolyzed casein; C, D, and H, casein and crystalline amino acids.

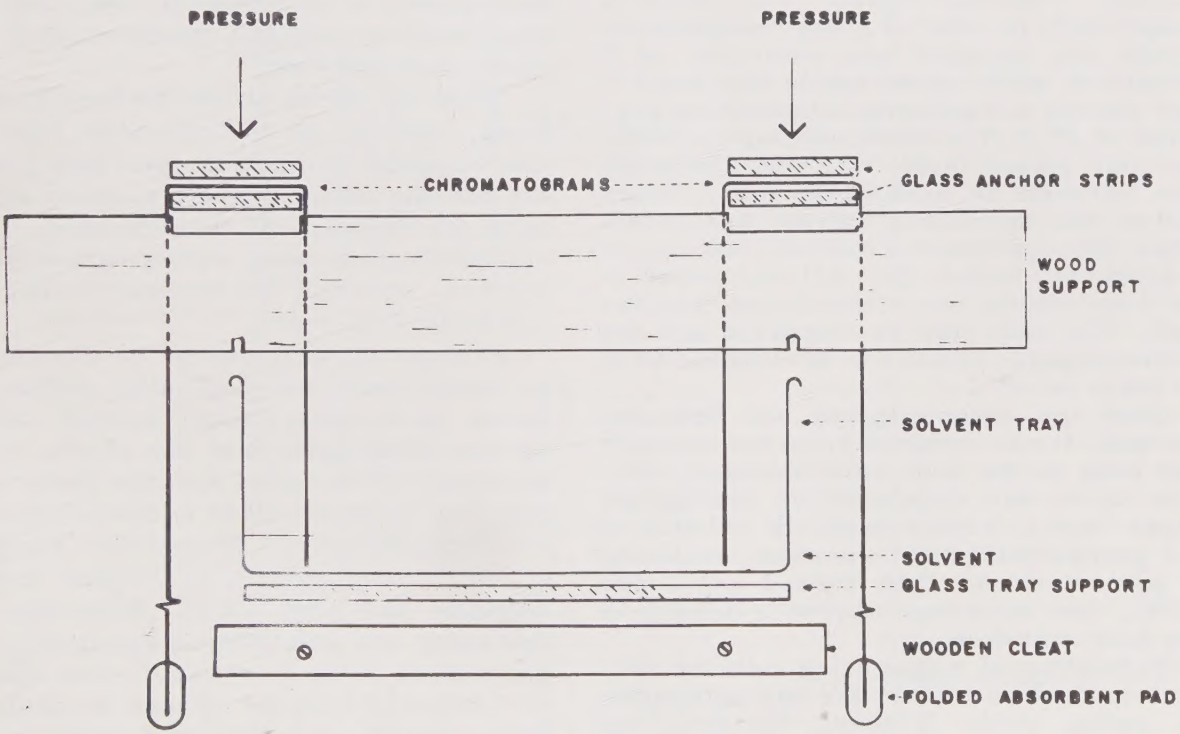

FIGURE 2.--Chromatogram suspension. Exploded view of solvent tray assembly showing method of suspending chromatogram. Note that wooden cleats are permanently mounted in chamber, supporting the glass plate for the aluminum solvent tray.

chamber, about 6 inches from the top, served to support the solvent assembly. An aluminum ice-cube tray, 4 by 11 by 1.25 inches, proved to be an adequate solvent tray. A small hole in the side of the chamber provided access to the tray for addition of the developing solvent. A sheet of ordinary window glass served as a covering and was sealed with silicone grease. The developing solvent consisted of buffered n-propanol. The buffer was prepared as follows: to 900 ml. of HOH were added 22.306 gm. of $Na_4P_2O_7 \cdot 10\ H_2O$, 3.28 gm. of NaCl, and 1.0N HCl to pH 7.3 (approximately 44 ml.) and made to 1,000 ml. A 75-25 ratio of n-propanol to buffer was routinely used. To saturate the atmosphere, 250 ml. of a 70-30 ratio of n-propanol and H_2O was placed in the bottom of the chamber.

In order to minimize possible fluctuations introduced by ambient temperatures, a constant temperature chamber, 33 by 39 by 38 inches, was constructed of 0.75-inch plywood and lined with sheet asbestos. The chamber was heated by approximately 125 feet of lead-armored heating cable, strung at 3.25-inch intervals through metal eyes along the four sides of the chamber, and a Fenwall "thermo switch" No. 47700-0, range 32° to 400° F. The temperature within the chamber was measured at 9 points--at each corner and in the center--and showed a maximum temperature gradient of 2° F. The chromatographic chamber was placed inside this box. The paper was allowed to equilibrate for 2 hours before the developing solvent was added. After the equilibration period, 300 ml. of n-propanol : buffer (75 : 25) was added to the tray via the small hole in the chamber wall. The hole was re-stoppered and the chromatogram allowed to develop for 44 to 48 hours at 30° C.

After the chromatogram had been developed, it was removed from the chamber and hung in the hood until thoroughly dry. The color was developed by dipping the paper into a freshly prepared solution of 0.2 percent ninhydrin in acetone containing 1 percent acetic acid (Roland and Gross 1954). The color was allowed to develop in the dark overnight.

Estimation of amino acids.--Of the several methods available for the estimation of amino acids (Fisher, Parsons, and Morrison, 1948; Naftalan 1948), it was believed that measurement of the maximum density of the spot directly on the paper held the most promise (Block, Durrum, and Zweig, 1958). For this purpose a Photovolt Densitometer Model 525, equipped with a 420-mμ filter, was used. The transmittance was adjusted to zero at the edge of the paper, and readings were made progressively up the paper strip. The maximum density of the unknown spot could then be interpolated on a curve derived from spots of known quantities of pure crystalline amino acids measured in a similar manner.

Results and Discussion

The results in this series of analyses are presented in tables 1 and 2. The figures represent the results of duplicate analyses, which were performed under conditions simulating those expected in a routine quality-control or pilot-plant program. For this reason, three replicates of five levels of each amino acid were used in the microbiological assays. Two diets were assayed on each chromatogram, and only three levels of each unknown and four levels of a known standard were spotted. These procedures have been shown to be practical and could be employed routinely in a quality-control or pilot-plant operation.

From the tables it can be seen that all three methods give comparable results. The variation between samples was greatest for the chromatographic assay averaging ±0.2 percent of content value. The microbiological assay values varied ±0.08 percent, whereas the enzymatic analysis varied ±0.07 percent.

It is not within the scope of this report to recommend one particular method of amino acid assay; each method offers certain advantages over the others. For example, the assay of a single amino acid may best be done either microbiologically or enzymatically. Enzymatic analysis is particularly adapted to routine assays (Seidman and Blish 1957). Materials for the assay are relatively inexpensive, and the method is the most rapid--this affords an additional saving. Where an analysis of the complete amino acid spectrum of a diet is desired, one of the chromatographic systems would be more applicable.

TABLE 1.--Comparison of essential amino acid content of casein by three methods of analysis

Amino acid	Reported values (grams amino acid per 100 grams protein) [1]	Observed values (grams amino acid per 100 grams protein)	Percentage recovery of amino acids added to casein	Observed values (grams amino acid per 100 grams protein)	Percentage recovery of amino acids added to casein
		Microbiological assay		Chromatographic assay	
Isoleucine---	5.4 - 6.8	6.15	100.7	6.07	100.1
Leucine------	8.5 - 10.6	9.16	103.0	9.78	101.3
Methionine---	2.9 - 3.3	1.49	104.8	1.77	95.1
Phenylalanine	4.7 - 5.4	5.29	98.0	5.45	104.6
Threonine----	3.5 - 4.5	4.12	103.2	3.94	95.5
Valine-------	6.2 - 7.7	6.93	100.8	7.01	101.8
Tryptophan---	1.0 - 1.5	.94	111.6	----	-----
				Enzymatic assay	
Arginine-----	3.8 - 4.3	4.3	105.1	3.75	101.1
Histidine----	2.6 - 3.4	2.76	101.2	2.90	99.2
Lysine-------	7.4 - 8.3	8.24	99.15	7.79	97.3

[1] Block and Bolling, 1951.

TABLE 2.--Amino acid analysis of two commercial fish diets

Amino acid	Diet No. 1		Diet No. 2	
	Observed values (grams amino acids per 100 grams protein)		Observed values (grams amino acids per 100 grams protein)	
	Microbiological assay	Chromatographic assay	Microbiological assay	Chromatographic assay
Isoleucine---	4.42	4.10	4.86	4.70
Leucine------	6.96	7.61	6.47	6.75
Methionine---	1.30	----	.84	----
Phenylalanine	4.36	4.10	4.23	4.35
Threonine----	4.01	4.24	4.06	4.14
Tryptophan---	.86	----	.96	----
Valine-------	5.88	5.44	5.68	5.43
		Enzymatic assay		Enzymatic assay
Arginine-----	5.87	5.62	5.22	5.38
Histidine----	2.44	2.54	2.25	2.17
Lysine-------	5.72	5.26	6.72	6.28

Microbiological methods of assay are relatively simple as compared to chemical methods of analysis and for many years have been the most commonly employed procedures for routine analysis. Block and Weiss (1956) emphasized the fundamental similarity of technique whereby the mastery of a single procedure enables the analyst to assay a number of vitamins or amino acids with only minor variation in general technique.

Perhaps the major disadvantage of microbiological assays is the time required to complete an assay. Usually a 72-hour incubation period is necessary, and an additional 2 to 3 hours is spent in titrating a normal series of a standard curve and four to five samples. Unless quite extensive facilities are available, at least 4 to 6 weeks would be expected for a complete assay of a diet.

It should be emphasized at this point that in one respect the simultaneous assay of several amino acids poses a problem not generally encountered. The wide range of amino acid levels found in most proteins and diets requires the selection of a compromise concentration of the hydrolysate. This concentration may not be optimal for all amino acids, and therefore some loss of accuracy must be expected. However, the loss of accuracy is not critical for most routine analyses and is adequately compensated by the increased number of assays possible in a given period. Most paper chromatographic procedures entail a developing time of 24 to 48 hours. To assay a chromatogram usually requires 0.5 to 1 hour of bench time, and an accuracy of ±10 percent can be expected (Block, Durrum, and Zweig, 1958).

Improvements in paper chromatographic methods are being made continually. Tuckerman (1958) reported a new procedure employing ion exchange resin impregnated paper that will resolve lysine, histidine, and arginine in 4 hours. Roberts, Kolor, and Bucek (1958) described a 2-hour procedure resolving tryptophan. A most elegant technique of amino acid analyses is ion exchange column chromatography as developed by Moore, Spackman, and Stein (1958). This method of analysis should be intensively studied in the very near future.

Summary and Conclusions

A series of amino acid assays was performed on standard casein and two commercially available fish diets. The results indicated that paper chromatography, either alone or in combination with enzymatic analyses, gave results which were comparable to microbiological assays in accuracy and were generally more rapid and economical to run in a quality-control or pilot-plant operation.

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Reporting on a study of the pirarucu, Dr. Osmar Fontenele of the Servico de Piscicultura, Fortaleza, Ceara, Brazil, noted that each fish of this species had but a single reproductive organ. Four females that were examined averaged 2 meters in length, and the weight of their four ovaries ranged from 495 to 1,300 grams. Four male specimens measuring 1.8 meters in length had a well-developed left testis but only a stunted right testis. One 1.88-meter male had a left testis measuring 260 millimeters and a right measuring only 25 millimeters.

Biologists are now using the number of pyloric caeca to distinguish between fingerling walleye and fingerling sauger.

RESULTS OF PLANTING KOKANEE SALMON IN TWO ADIRONDACK MOUNTAIN LAKES, NEW YORK

Dwight A. Webster and William Flick
Cornell University
Ithaca, New York

KOKANEE OR DWARF SOCKEYE SALMON (Oncorhynchus nerka) are planted in several New York lakes to provide angling (Greene 1952). In addition to providing angling directly, kokanee may also serve as an important forage fish for other salmonids in many western North American lakes (Larkin 1954). This note describes the results of experimental plantings of advanced kokanee fry in two lakes, in one of which the aim was to observe the possible use of this species as a forage fish for landlocked salmon (Salmo salar).

The source of the kokanee stock was Flathead Lake, Montana. Eyed eggs representing three year-classes were hatched at the Cornell University Experimental Fish Hatchery. The 1955 year-class was planted as advanced fry shortly after the fish began feeding (May 4, 1955), whereas the 1956 and the 1957 year-classes were planted as 1-inch fingerlings late in May or early in June. Fish were released in the several "schools" in the open waters of the lakes.

Black Pond

Black Pond is on the Brandon Preserve in Franklin County and has an area of 25 acres and maximum depth of 58 feet. The pond is thermally stratified; a severe oxygen deficit develops by late summer. The watershed is limited, and the outlet is intermittent and goes underground a short distance from the discharge.

In addition to landlocked salmon and kokanee, the fish population consisted of brook trout (Salvelinus fontinalis), lake trout (S. namaycush), common sucker (Catostomus commersonni), brown bullhead (Ictalurus nebulosus), pumpkinseed (Lepomis gibbosus), and minnows (Notropis cornutus, Chrosomus eos, and Pimephales notatus). Alewives (Alosa pseudoharengus) were introduced in the spring of 1958; although eradication of the fish population in Black Pond was scheduled for the fall of 1958, the planting provided an opportunity to obtain preliminary data on the establishment of this species as a forage fish (Vincent 1960).

Three year-classes of kokanee (20,000 to 40,000 per year) were planted, the work beginning in May 1955. The evaluation of the success of these plantings is based on trap netting in the fall of 1957 and gill netting in the spring of 1958, as well as on a rotenone sample in the fall of 1958. Only three kokanee were taken by angling, but pressure directed toward capture of the species was negligible.

The 1955 year-class of kokanee salmon matured in part in the autumn of 1957 at age II+. A trap net fished November 3-12 took 40 mature males and 9 green females. Mean lengths and weights are shown with the size distribution in figure 1. The largest individuals were males of 14.2 inches and 0.9 pound. Two juvenile fish of this year-class (11.2 inches) were also taken, forecasting divided age at maturity. The sample included five juvenile kokanee

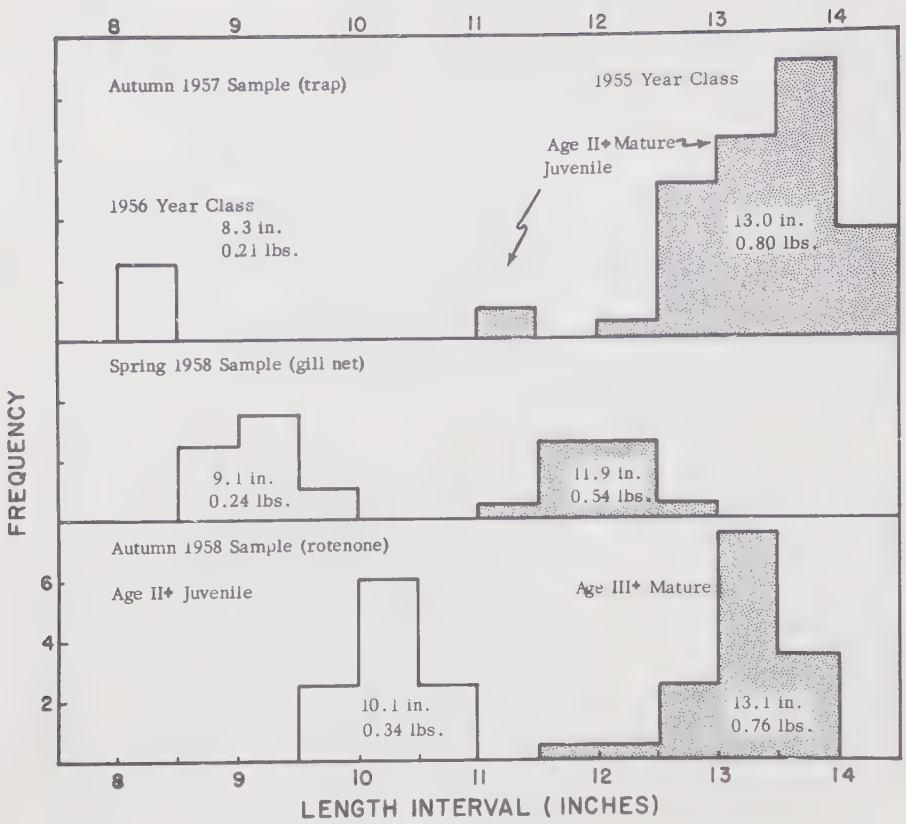

FIGURE 1.

of the 1956 year-class (age I+) that averaged 8.3 inches and 0.21 pound.

A gill-net sample on May 18, 1958, caught 27 kokanee of these 2 year-classes (figure 1). The 1955 year-class, now age III+, was represented by fish that did not mature in 1956. This group was only 11.9 inches, compared with 13.5 inches for the maturing individuals the preceding autumn, indicating that it was a slower-growing fraction of the year-class that did not mature at II+.

Mature age III+ members of the 1955 year-class were about the same size (13.1 inches) as II+ mature fish. Of this group, 186 were recovered during the rotenone application of October 4, 1958.

The balance of the rotenone sample was 252 kokanee of the 1956 year-class (age II+). A sample of 23 specimens averaged 10.1 inches and 0.56 pound; thus a marked reduction in growth appeared when these kokanee were compared with the previous year-class. Some larger members may have matured and would be included with mature salmon grouped together as age III+ of the 1955 year-class. Size distributions in figure 1 do not suggest this; and the presumption is that most, if not all, of this year-class would not have spawned at age II+.

Total weight of recovered kokanee was 227 pounds, or a minimum standing crop of about 9 pounds per acre. Probably the number of kokanee recovered represents close to the maximum present, although there is no objective means for judging this. Only 12 dead kokanee were noted on the following day. All of these were resting on the bottom, so specimens in deep water would escape detection. 1/

The reaction of kokanee to the rotenone was unusual and would tend to make a high recovery possible. It deserves comment in view of the low recoveries of fish normally made from deep lakes reclaimed when surface water temperatures are cold (57° F. in this instance).

The sequence of rotenone application was as follows: The shallow outlet bay was treated first (figure 2), and a predominant

1/ Intensive gill netting for a week prior to application of rotenone gave no indication of the density of the kokanee stock. Only one was taken, despite good catches of other larger species in the lake. Kokanee were off bottom and well off shore during this time.

number of the minnows and sunfish were killed. The bulk of the chemical was applied by spray bar between 9 a.m. and noon; the work was begun at the northeast end of the pond and was continued systematically to the southwest end. Only an occasional kokanee appeared until midafternoon, despite prominent mortality in other species known to occur in the lake. From midafternoon on, however, there was extensive activity of distressed kokanee--but in a limited area at the southwest end (shaded area, figure 2). It appeared that the kokanee were driven by an advancing curtain of toxic water until concentrated here. The severe reduction in dissolved oxygen, from 4.0 p.p.m. at 25 feet to 0.5 p.p.m. at 30 feet, doubtless figured prominently in producing this effect. Moribund fish surfaced repeatedly and were rapidly collected until late afternoon, when activity declined markedly.

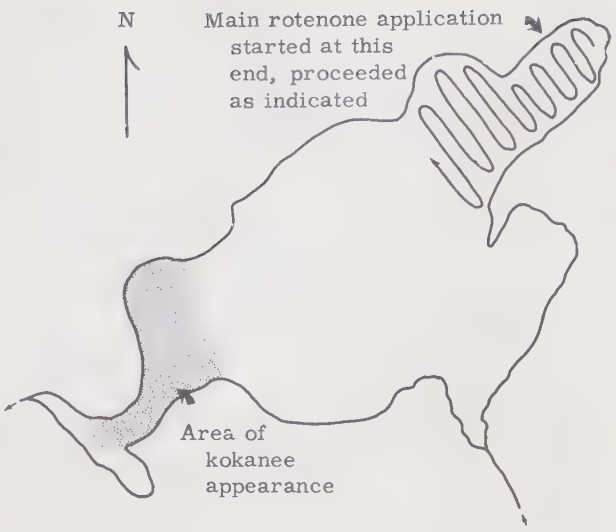

FIGURE 2.

No recoveries of the 1957 year-class were made, and it is presumed that this planting was a complete failure. These fry were in poor condition after transportation. There was probably considerable predation on the newly planted fry, as landlocked salmon taken by angling on the day after the planting held large numbers.

In addition to the planted year-classes, there were seven recoveries of fingerling kokanee of the 1958 year-class that was naturally spawned in Black Pond. These fish averaged 2.5 inches. Spawning areas are unknown, but the extensive gravel shoal where adults were trap-netted in 1957 is a likely choice. The inlet to Black Pond has a low gradient and flow and is accessible only to small fish.

The initial planting of landlocked salmon was 1,000 fry in June 1953. This was followed in June 1954 by a planting of 250 3-inch yearlings (same year-class) that were fin-clipped. The main angling catch was made in 1957, when 42 salmon were taken, all except 1 fish being of the planting of marked yearlings. The mean length of 28 salmon removed was 17.3 inches; the weight, 1.2 pounds. Nine mature salmon trap-netted in the autumn of 1957 (age IV+) averaged 16.9 inches in length and 1.3 pounds in weight. In 1958, a severe deterioration in condition was indicated by a mean weight of 0.81 pound for fish of essentially the same length (17.2 inches) as the preceding year.

Third Bisby Lake

This lake is one of a chain of four Bisby Lakes located on the Adirondack League Club, Herkimer County. The area is 44 acres, and the maximum depth is 40 feet. The water is yellow-brown, and an oxygen deficit occurs at depths below 35 feet. The lake contains lake trout, a remnant population of frostfish (Coregonus cylindraceum quadrilaterale), and smallmouth bass (Micropterus dolomieui), as well as considerable numbers of suckers.

A single planting of 5,000 kokanee was made May 4, 1955. No fish were taken by angling until the 1958 season, when two were taken on spinning lures and others were hooked. This lake is relatively inaccessible, and fishing pressure was so light as to provide an indeterminate test of availability to angling; however, several parties made special efforts to take the fish, using methods known to be successful in other waters.

Observations and netting for spawning fish were conducted in the autumn of 1958.

No fish were observed on November 1; but on November 21 four pairs of kokanee were seen spawning in a limited area of the inlet, a short distance from the lake. There was no indication of lake spawning along the limited shoreline where gravel was available. Eleven kokanee seined from the spawning group or gill-netted from the lake were substantially larger than the Black Pond fish, averaging 17.3 inches (range in length, 16.8 to 18.0 inches) and 1.8 pounds. All females were gravid or nearly so, and some were partly spent. In view of divided age at maturity in Black Pond, it is assumed that some spawning of the 1955 year-class in Third Bisby Lake also occurred in 1957. A few salmon reached an age of IV+ and presumably spawned in the fall of 1959. Scales from one such fish taken in July 1959 showed four annuli, the last annulus (1958-59) exhibiting some erosion.

The stream area that was selected by the spawning fish was of poor quality, but as good as was available in the inlet brook. The bottom was mostly large, well-consolidated rubble, from which only imperfect redds could be constructed. A large number of eggs were lying free on the bottom below the spawning groups, indicating failure to lodge properly in the shallow egg pits.

Discussion

The diverse size attained by kokanee adults of the 1955 year-class planted in Black Pond and Third Bisby is doubtless a reflection of different levels of competition and perhaps of basic productivity. The parental stock in Flathead Lake (also introduced) averaged about 13 inches in length in 1952, presumably at the same age (Stefanich 1952). No data on divided maturity under stabilized conditions in this population are available.

The Black Pond test was inconclusive in determining the value of kokanee as food for landlocked salmon. Greater efficiency of kokanee in using plankton probably contributed materially to the rapid deterioration of the condition of the landlocked salmon already present. This has been observed in some west-coast lakes where kokanee were introduced as forage for rainbow trout, particularly in small waters (Larkin 1954). It is possible that planting substantially larger numbers of fry, if accompanied by sufficiently high levels of survival, would have produced desirable stunting and lengths of 6 to 8 inches at maturity. In Black Pond stunting was in progress at the termination of the study, and a full complement of age-groups 0 to III would doubtless have had a marked effect on growth.

From the standpoint of management, maintenance of a forage species by artificial plantings would not be practical. The spawning conditions for kokanee might be improved in Black Pond, but it appears doubtful that even under optimum conditions they would be adequate to maintain a dense stock of kokanee.

The future program for Third Bisby Lake consists of improvement of the natural spawning area and additional plantings of fry, with a view to establishing a self-perpetuating population. Although several successful introductions of kokanee have been made in New York, maintenance of the stocks is dependent on annual plantings. One Connecticut lake maintained a natural population for several years (Webster 1940), but it became extinct when efficient angling methods were developed.

Acknowledgments

The interest and the cooperation of Mr. Walter W. Allen, Superintendent of Fisheries, Montana Department of Fish and Game, and the Montana Fish and Game Commission in supplying kokanee eggs are appreciated. The Adirondack League Club and Mr. and Mrs. Donald P. Ross of Montchanin, Delaware, made these two ponds available for experimental planting. Cornell graduate students--Robert Vincent, James Reckahn, Henry Regier, E. Wayne Shell, and Marvin Grosslein--assisted in the Black Pond reclamation, as did also R. N. Kimpton, Jr., Roy Hungerford, Edward Osier, and Roy Duprie of the Brandon Preserve staff. Robert Blake

and Peter Bertine assisted in the collection of the Third Bisby Lake samples. Fish-cultural parts of the study were conducted by Mr. C. W. Lyon, Jr., fishery technician, Cornell University.

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A PORTABLE CONDUCTIVITY METER FOR USE WITH ELECTROFISHING GEAR

For the water-conductivity determinations that should precede electrofishing work, we have modified a Bouyoucos BN-2 transistorized moisture meter (Industrial Instruments Company, Cedar Grove, New Jersey). We have masked out all but the measured-resistance scale on the face and attached two clips to the meter case to hold the dip-cells. The leads of the cells can be permanently attached to the terminals on the meter.

One dip-cell has a constant of 0.1 and is used in highly resistant waters; the second has a constant of 1.0, is used in less resistant waters. The meter's range is from 100 to 300,000 ohms, with 2,500 and 25,000 ohms falling in the center of the scale. Between 100 and 50,000 ohms, the range of most North American fresh waters, accuracy is well within 10 percent of the scale reading.

The meter is powered by four pen-light batteries--cheap, available everywhere, and reported to last an average of 2 years.

To determine water conductivity, the cell is put into the water. (Care should be used, especially in swift water, to see that there are no bubbles in the cell to give an erroneous high reading.) The ON button is pressed, the meter needle adjusted to zero. The TEST button is then held down, and the resistivity of the water is read directly from the dial, in ohms per cubic centimeter of water.

This meter is portable, light in weight, small (3 by 4 by 6 inches), and durable. The conductivity of water can be determined much faster with this meter than with that described by R. E. Lennon ("The Electrical Resistivity Meter in Fishery Investigations," Special Scientific Report--Fisheries No. 287), and the less cumbersome instrument costs about half as much as the other.

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INFECTIOUS PANCREATIC NECROSIS OF TROUT
I. A TISSUE-CULTURE STUDY

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INFECTIOUS PANCREATIC NECROSIS is an acute high-mortality disease which has been most frequently observed in young eastern brook trout (Salvelinus fontinalis). It has been found occasionally in rainbow trout (Salmo gairdneri) and brown trout (Salmo trutta) and might yet be found in other young salmonids. Wood, Snieszko, and Yasutake (1955) were the first to characterize this disease as an infectious pancreatic necrosis, and this, originally a descriptive phrase, has since been generally accepted by fish-pathologists as the name of the disease. According to that publication and to another by Snieszko, Wood, and Yasutake (1957), this disease is probably the same as acute catarrhal enteritis, which was described by M'Gonigle in 1941.

The histopathological changes brought about by the disease have been described (Wood et al. 1955; Snieszko et al. 1957). The former work included some evidence of the infectious nature of the disease, but the authors were not able to find bacteria or other parasites. They did find inclusion bodies in the lesions of the pancreas. For these and other reasons, they postulated that the disease was caused by a virus. The most recent work of Snieszko, Wolf, Camper, and Pettijohn (in press) clearly demonstrated its highly infectious nature.

The purpose, therefore, of the work reported here was: (1) to find susceptible tissues and suitable methods to permit isolation of this agent from diseased trout and to permit cultivation of this agent in vitro, (2) to test the hypothesis of a virus cause, and (3) to determine, if possible, some of the attributes of the agent which caused the disease.

Preparation of Cultures and Inocula

Today, many animal viruses have been successfully propagated in and otherwise studied in tissue culture. Once the particular requirements have been met and methods determined, tissue culture has usually proved to be a more versatile and convenient method of research than use of the normal host. So far, infectious pancreatic necrosis is known to occur only in young trout. In addition to being convenient, a susceptible tissue-culture system frees the laboratory from the limitations of the short seasonal availability of small fingerling trout. For these reasons, methods of tissue culture formed the basis of study about which this phase of the research revolved.

At least two additional reports on this disease will appear. The second paper in the series will be a report on the infectivity trials conducted with eastern brook trout during the last season. The third paper will be a report on the attempts to show the degeneration of infectious pancreatic necrosis virus in cultures or cells of mammalian origin, and the attempts to determine whether this virus was capable of hemagglutination.

Explant-type cultures of juvenile or adult trout tissues were set up according to methods and media described by Wolf and Dunbar (1957). The explant-type culture consists of small pieces of tissue attached to an inside wall of a glass vessel. Human cord serum at a level of 20 percent by volume was most often used as the nutrient portion of the media, but in some trials chicken or human serum or bovine amniotic fluid was substituted. Antibiotics were added to all media to suppress bacteria and molds that may have been present in or on the fish from which the tissues were taken or which accidentally contaminated the cultures during handling. The usual concentration was 450 units each of penicillin and streptomycin and 50 units of mycostatin per milliliter of medium. All the cultures of fish tissue were incubated at 19° C., and the cultures were allowed to form extensive cell sheets, that is, to "grow" for several days before they were inoculated.

The HeLa cell line, a well-known and widely used transferable cell of human malignant tissue origin, was propagated at 37° C. in 80 percent synthetic medium 199 and 20 percent horse serum with antibiotics.

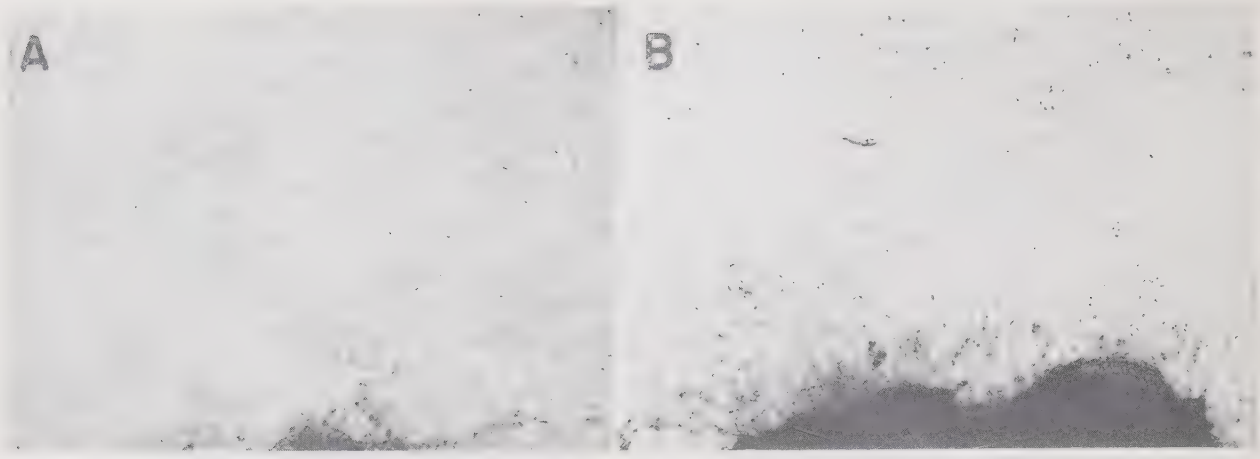
Inocula of pancreatic necrosis were prepared from moribund specimens of fish presumptively diagnosed as having the disease. Some inocula were prepared from fresh specimens, others were prepared from fish which had been frozen and held at -20° C., and still other inocula were prepared from diseased tissue that had been held in 50 percent glycerin at 4° C., a standard treatment for preservation of live virus. Specimen fish were ground in several times their volume of tissue-culture salt solution. The pulp was then sedimented for 10 minutes at 10,000 or 15,000 revolutions per minute. The clear supernatant fluid was filtered through bacteria-retaining asbestos or porcelain filters. Cultures were usually inoculated with 0.01 to 0.1 milliliter of the material so prepared, but for some purposes the inocula were serially diluted.

Observations

Outgrowths of cells from explants of the swim bladder, spleen, kidney, gills, and caudal fin of eastern brook trout were consistently attacked by filtered bacteria-free inocula prepared as described from trout presumptively diagnosed as having infectious pancreatic necrosis. Every epizootic thus far checked has yielded fish from which the inoculum has produced this degeneration, but inocula prepared from healthy trout produced no such degeneration. Visible change in the inoculated cultures was often observed within 18 hours, and complete destruction of the tissue outgrowth often occurred within 2 days, but at times 5 or more days elapsed before destruction was complete.

Explants prepared from caudal fin were free of fatty tissue and could be prepared with a minimum of cellular debris. This was the easiest tissue to obtain, and its rayed structure permitted the cutting of very uniformly sized pieces. At 19° C., these explants, or pieces of tissue, rapidly gave rise to sheets of uniform, epithelial-like cells which were susceptible to the degenerative changes brought about by inoculation with pancreatic necrosis. For these reasons, explants of caudal fin were the preferred tissue for use in this study, and it was in such cultures that the following sequence of events was usually observed.

The first difference that could be noticed in living inoculated cultures was a darkening of some of the epithelial-like cells. This typically occurred first at the periphery of the outgrowth, but a simultaneous appearance of "islands" of such cells was also observed in the otherwise normal-appearing cell sheets. The darkening was not pigmentation but was probably a result of contraction and increased density of the affected cells, as it was typically followed by a breakup of the sheet. This breakup was much like the usual effects of a cytopathic virus in tissue culture. It looked much like the destruction of certain human cell cultures brought about by Herpes simplex, the "cold sore" virus, illustrated in the



Infectious pancreatic necrosis produced striking changes in test-tube cultures of certain fish tissues. (A) Uninoculated control culture of caudal-fin tissue of eastern brook trout. An intact sheet of epithelial cells extends outward from the explant, a corner of which is visible at bottom center. (B) An inoculated culture of fin tissue from the same fish. This culture's epithelial sheet was destroyed by the agent of infectious pancreatic necrosis. A few wisps of degenerate epithelial cells extending across the top of the area were all that remained. Spindle cells were less rapidly attacked, and such cells can be seen projecting from the edge of the dark explant at the bottom. Hematoxylin and eosin stain. About 390X.

report of Lerner, Takemoto, and Shelokov (1957). After the appearance of the darkened cells, adjacent cells withdrew from each other and became free of the vessel wall. A rather characteristic cell shape was evident at this time: the outline of this cell resembled an elongate football or the top view of a canoe. As destruction progressed, nearby areas of necrosis enlarged and coalesced. Other areas of necrosis coalesced with the line of destruction, which moved inward from the edges. In time, only tattered remnants of the formerly intact cell sheet remained, but these remnants eventually disappeared, and ultimately only the explant itself remained (see figure). Control cultures were not so affected (see figure). Spindle or fibroblast-like cells, which were slower to appear from explants, were more resistant to changes brought about by inoculation but were not immune.

As in the work with brook trout tissue, cell sheets which developed from rainbow trout caudal-fin explants proved to be susceptible to the cytopathic effect evoked by infectious pancreatic necrosis. Thus far, cell sheets from brown trout tissue have proved to be refractory. In a single trial, cell sheets of bluegill (Lepomis macrochirus) caudal-fin explants were similarly unaffected by an inoculum of proved potency. HeLa cells inoculated with infectious pancreatic necrosis filtrates and incubated at 19°, 25°, or 37°C., similarly displayed no cytopathic changes.

It is a common practice to leave an inoculum of virus in a tissue-culture vessel until the nutritive fluid is spent and requires change. This keeps the virus and the cells in continuous contact for several days. In this study such a practice was usually followed, and as a result the cell sheets were in continuous contact with the inocula for 3 or more days. This was largely a matter of convenience, but a far shorter exposure to the inocula produced comparable degeneration. For instance, when inocula were allowed to remain in culture vessels for as short a period as 15 or 30 minutes, after which the explants were repeatedly washed with tissue-culture salt solution, there were

cytopathic changes which were comparable to those produced in parallel cultures that had been inoculated but not flushed.

The agent of tissue-culture degeneration proved capable of at least limited transfer, but such transfers were accompanied by diminishing destruction in the later transfers. Two such culture lines of the agent were transferred eight consecutive times. By this process the eighth transfer could contain only 1/25-billionth of the inoculum used in the first tissue culture. Multiplication of the agent must have taken place during the successive transfers, because much lower dilutions of such original inocula had no visible effect on inoculated cultures of trout tissue. In the eighth transfer the tissue degeneration was still present but was less extensive and not typical.

Inocula were prepared from diseased fry that had been frozen and held at -20° C. for as long as 4.5 years, and from other fry that had been preserved in 50 percent glycerin at 4° C. for 2.5 years. In both instances the ability to produce degeneration remained.

After inocula were heated at 60° C. for 1 hour, they did not produce cytopathic degeneration.

Discussion and Conclusion

In a suitable tissue-culture system, many of the animal viruses have been shown to cause cytopathic changes. However, tissue cultures are highly sensitive biological entities, and similar-appearing cytopathic changes can be evoked by toxic substances. The results of this work show that bacteria-free material from trout which died of infectious pancreatic necrosis consistently caused degeneration in trout-tissue cultures. Such degeneration could have been caused by either a virus or toxic material present in the diseased trout. That such toxicity was not present and did not cause the degeneration produced in the cultures used in this study was evidenced by the fact that bluegill and brown trout tissues and the HeLa cell line were unaffected by filtrates which produced degeneration in parallel cultures of susceptible fish tissue. Considering, too, the dilutions that accompanied the series of eight tissue-culture passages, it is again most unlikely that the degenerative changes were caused by a toxic substance present in the inocula.

Infectivity of these inocula was not eliminated by filtration through bacteria-retaining filters. Relatively high concentrations of antibiotics were present in all the cultures used in this study, and these antibiotics did not eliminate the ability of the inocula to produce degeneration. Similarly, the preservation of infectivity in 50 percent glycerol--a characteristic of the agent reported here--is only rarely encountered among bacteria.

Heat inactivation at 60° C. for 1 hour is a common practice in virological studies, and the agent reported here was completely inactivated by such treatment.

The decrease in cytopathogenicity that accompanied repeated transfer had many of the characteristics of attenuation or decrease in virulence that occurs when some microorganisms are propagated in artificial culture.

As studied in tissue culture, pancreatic necrosis of young trout was a transferable and infectious disease caused by a filterable agent which had the ability to remain viable in 50 percent glycerin; was unaffected by penicillin, streptomycin, and mycostatin; produced characteristic cytopathogenicity in some tissues; and was heat-labile. It was concluded, therefore, that the disease was caused by a virus.

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PLASTIC WADING POOLS USED AS PORTABLE CIRCULAR REARING TANKS FOR SALMON FRY

In the winter of 1958-59 at the Kitoi Bay Research Station, plastic wading pools--8 feet in diameter, capable of holding 20 inches of water--were set on wood platforms standing 18 inches from the floor and used as rearing tanks for salmon fry.

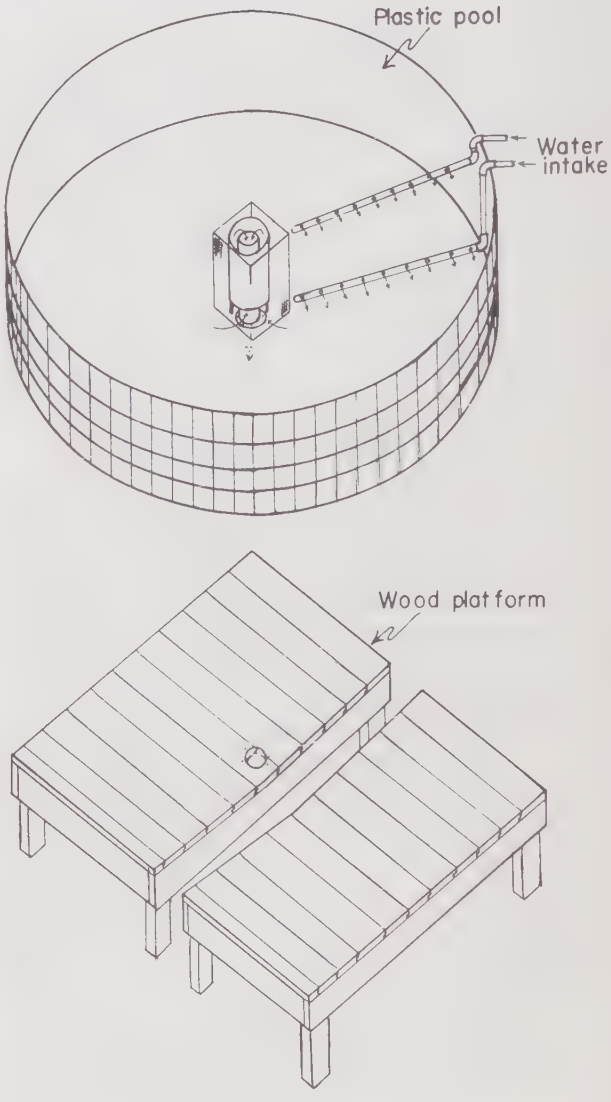
Each platform was built in two matching 4- by 8-foot sections. The "inside" 8-foot beams in each section were sawed so that they were 2 inches less in depth at the center than at the ends. Across inside and outside 8-foot beams, 4-foot pieces of 1-inch shiplap were nailed. Set together, two sections made a square platform with its center 2 inches below the outer edges.

Through a 4-inch hole cut in the center of the pool bottom and a matching hole in the supporting wood, a threaded iron floor flange was passed and fastened to the platform with lag screws. This was a solid base for a 3-inch (inside diameter) iron standpipe--threaded, cut to any desired length, and easily removed for cleaning.

Water entered from pipes perforated on one side: one pipe in a "radius" position on the pool bottom, another on the surface. A pipe of 8-inch diameter, on three 1/2-inch legs, was set around the standpipe to force water entering it to rise from the bottom. A box screen, 6 meshes to the inch, kept fry from entering the standpipe.

Initial use has shown that, with some modification, the device has real promise.

--WILLIAM A. SMOKER and SHERMAN W. MARBLE, Alaska Department of Fish and Game, Juneau.



COST IN RELATION TO SIZE OF MONTANA'S HATCHERY TROUT

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THE NUMBER OF SEVEN-INCH OR LARGER TROUT planted annually from State-operated hatcheries in Montana has increased steadily from 68,000 in 1948 to 675,000 in 1958 (figure 1). Furthermore, the average size of these fish has increased from 7.5 inches to 8.5 inches during the last 7 years for which size records are available. Obviously, these large fish cost more than fry and fingerlings. To determine precisely how much the large fish cost in relation to costs of smaller trout was the main objective of this analysis. Without this information, it is impossible to determine the cost of creeled hatchery trout; hence no decisions can be reached as to what sizes of fish are most economical to plant in the various management situations.

The Montana Fish and Game Department operates 12 trout hatcheries, 1 pike hatchery, and a varying number of spawn-taking stations. Excluded from this report are the cost and production of the pike hatchery, an annual allocation of funds to four Federal hatcheries located in Montana, production of the Federal stations, and central-office administrative costs.

The annual cost of operating the State trout hatchery system has ranged from $232,000 to $396,000 for the last 10 years. The trend has been toward progressively higher expenditures, but the rate of increase has not been excessive in relation to the increase for the entire Fish and Game Department. For example, the total hatchery expenditure for the fiscal year ending in 1953 was $277,000, and in 1958 it was $396,000, a 43-percent increase. Expenditures by the entire Fish and Game Department increased from $1,281,000 to $2,427,000, or 89 percent, during the same 5 years. Of the Fishery

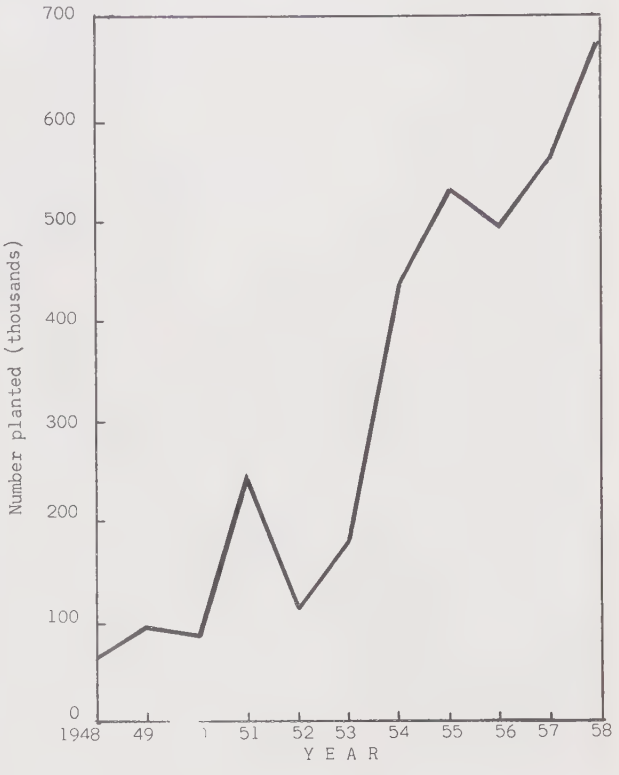

FIGURE 1.--The steadily rising trend in the numbers of 7-inch or larger trout planted during the period 1948 through 1958.

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Division expenditures, 90 percent was spent for the hatcheries in the fiscal year 1948-49, and this gradually declined to a record low of 51 percent in 1957-58. Most of the difference has been spent on purchases for fish habitat, fisherman access, and rehabilitation of trout waters. Hatcheries are still the most expensive tool of fishery management, and accurate cost analyses are necessary to insure proper control of this facility.

The numbers of fish planted annually during the last 10 years have ranged from 9 million to 20 million, with no prevailing trend toward higher or lower numbers. The total number of fish planted each year is determained mainly by availability of eggs and need for small fish in lakes and major rehabilitation projects. Species planted from State-operated trout hatcheries are: rainbow trout, cutthroat trout, golden trout, brown trout, brook trout, lake trout, kokanee, silver salmon, and grayling.

Hatcheries of the Montana Fish and Game Department use the Fish and Wildlife Service's method of hatchery bookkeeping and annual reporting, described by Tunison (1957). Unfortunately, the annual report does not give a breakdown of the cost of rearing and planting trout in in relation to size. Therefore, supplemental calculations were used to estimate the cost of hatchery trout of any given size by 1-inch class intervals. The average cost per fish planted was computed for each trout hatchery. Because some hatcheries plant fry exclusively, while others gear their production to maximum output of catchable fish or fingerlings of varying sizes, it was possible to rough out a graph depicting average cost per fish in relation to size of fish. By using 4 years of cost and production data, many points were established on the graph. Up to the 3-inch size, the points fell close to a hypothetical line representing average cost per fish. Hatcheries specializing in catchable trout also plant widely fluctuating numbers of smaller fish from year to year; hence the relationship between size and cost was not clearly defined beyond the 3-inch size class. It was necessary, therefore, to verify the estimated costs of larger sizes by another method. As it was possible to calculate the weight gain necessary to rear fish from the 3-inch to the 4-inch size class, from the 4-inch to the 5-inch, etc., and accurate cost-per-pound data were available from the annual reports, it was simple arithmetic to progress from cost per fish at one size class to cost per fish at the next size class. This was done for sizes up to 10 inches (see table). By way of checking the accuracy of these cost estimates, the numbers of the fish that had been planted in 1956 and 1957 were multiplied by the appropriate costs at 1-inch class intervals. The total values thus obtained were $330,000 for 1956 and $330,500 for 1957. There may be some question as to how these values might be compared. It is obvious that a 10-year average ($308,000) is too low because of the indicated trend toward higher operating costs of the hatchery system. The average expenditure for these 2 years, 1956 and 1957, was $327,000. This is within 1 percent of the total values calculated from the cost estimates in the table. Actual total expenditures for operating the hatcheries were $261,000 for the fiscal year 1955-56 and $392,000 for 1956-57. Therefore, it appears that the estimates

THE TOTAL COST OF REARING AND PLANTING FISH OF ANY GIVEN SIZE UP TO 10 INCHES IN LENGTH FROM MONTANA FISH AND GAME DEPARTMENT TROUT HATCHERIES, 1956-57

Size (inches)	Cost per fish	Cost per pound
Eggs	$0.003	---
Kokanee and grayling fry	.003	---
1	.005	$12.72
2	.010	3.04
3	.023	2.03
4	.048	1.81
5	.09	1.77
6	.14	1.62
7	.22	1.62
8	.33	1.63
9	.47	1.63
10	.64	1.62

of cost per fish in relation to size are accurate enough for management purposes.

It is of prime concern to anyone who is analyzing trout-production costs to know exactly what expenses are included. The costs presented in the foregoing portion of this report and in the table are total costs based on the philosophy that all the money allotted to the fish-hatchery portion of the Montana Fish and Game Department should be charged against fish production. Tunison (1957) listed the costs that are included in the "cost of production" under the Federal cost-accounting system and formally adopted by the Montana Fish and Game Department in 1957. Tunison stated that, on the average, 60 percent of the funds allotted to hatcheries are used for producing fish and the remainder is spent on such items as "new construction, 'park' maintenance, experimental work, hatchery assistance given other activities in the agency, central-office overhead, and amortization of the facilities." The percentage charged to production will be higher for large hatcheries than for small hatcheries. The percentage of funds charged to production of fish at Montana's State-operated hatcheries has ranged from 25 percent to 71 percent and averaged roughly 50 percent. This is lower than the 60 percent reported by Tunison for Federal hatcheries and is probably a reflection of the fact that several of the State hatcheries are quite small in comparison to the Federal hatcheries reported by Tunison (1957). The annual production of 7 of the 12 State-operated trout hatcheries seldom exceeded 8,000 pounds (range: 300 to 9,000 pounds). The smallest hatchery that was described by Tunison had an annual production of 8,000 pounds. Also, the difference is partly due to the fact that new construction costs have been included in State reports (but not charged against production), whereas Federal hatcheries exclude certain new construction costs from their reports. To convert the cost-per-fish and cost-per-pound estimates in the table to "cost of production" for comparison with Federal hatcheries or any other hatcheries using the same system of accounting, it is necessary to divide the total costs by 2. For example, 7-inch fish cost 22 cents each to rear and plant, according to the table, but only 11 cents each if the total cost is converted to "production cost." The average production cost for Montana trout hatcheries in 1957 was 84 cents per pound.

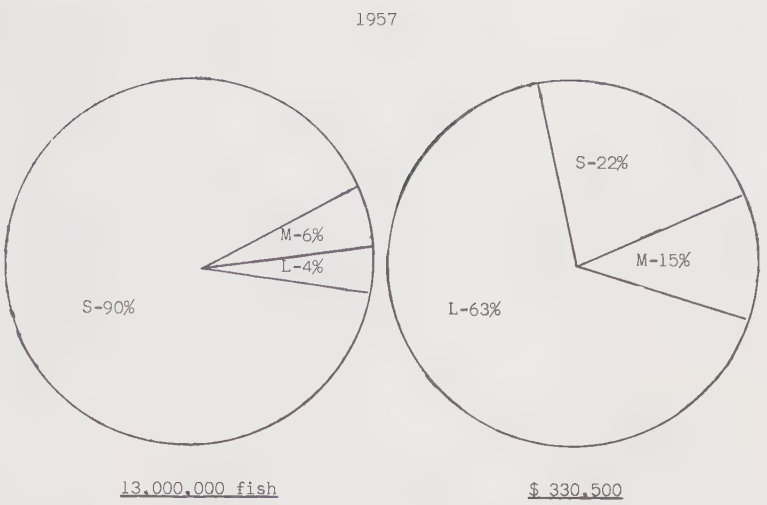

FIGURE 2.--Pie charts illustrating the large amount of money necessary to rear and plant only a small fraction of the fish as catchables. (S = eggs, fry, 1- and 2-inch fingerlings; M = 3-, 4-, 5-, and 6-inch fingerlings; L = 7-inch or larger fish.)

In 1957, 4 percent (563,362) of the 12,700,000 fish planted were 7 inches long or larger. Using the appropriate cost estimates from the table, these catchable fish accounted for 63 percent ($207,480) of the $330,500 total value of fish for that year (figure 2). This does not mean that 63 percent of the hatchery budget could have been diverted to other forms of fishery management had no catchable fish been reared. The only savings would be in the food and distribution costs of the catchable fish. In this hypothetical instance, since the food costs were roughly

30 cents a pound and distribution costs about 8 cents a pound at hatcheries where most of the catchable fish were reared in 1957, the savings would have amounted to $42,528 for food ($0.30 x 141,760 pounds of catchable fish planted) and $11,341 for distribution ($0.08 x 141,760 pounds). It is necessary to assume that the fish that were planted as catchables would not have been reared at all to achieve these hypothetical savings ($53,869), which amount to only one-fifth of the total estimated value of the catchable fish ($207,480). The savings would be even less if it were assumed that the fish would be reared to some subcatchable size. The remainder of the difference between theoretical savings and estimated value of the fish would, in all cases, have to be absorbed in higher estimated values for the subcatchable sizes. This leads to the dangerous fallacy that catchable-size trout are a byproduct which can be produced at little extra cost because the labor force, plant, and equipment are already there for the purpose of producing subcatchable fish. The author does not subscribe to this, but believes that catchable fish should share alike with smaller fish in justification for the hatchery and its operation.

Two factors are now operating to induce fish-culturists to produce increasingly larger numbers of increasingly larger trout. First, there is the emphasis on cost per pound of fish produced as the single most significant figure in judging hatchery efficiency. Second, there is the fact that to produce at peak efficiency, hatcheries must operate at full capacity the year around (Kingsbury 1951, Tunison 1957). Public sentiment is easily swayed to support the catchable-fish program because of its immediate positive yield to the creel. Consequently, the catchable-fish program continues to grow without clear demonstration of need or superiority over other management tools. Indeed, the fishery manager who requests large numbers of small trout and but few catchable trout finds himself unpopular with fish-culturists who are competing to produce pounds of fish as cheaply as possible. Tunison (1957), however, stated that "capacity production at a hatchery may not be required for management purposes and this fact should be recognized in evaluating its production record." To this we should like to add that "cost per creeled fish" should be as important as, if not more important than, "cost per pound" in the evaluation. Due consideration, of course, must be given to size and quality of the creeled fish. The analysis of cost in relation to size of fish presented here is a necessary step in this direction. Ultimately, it may be desirable to make separate cost-in-relation-to-size estimates for each hatchery. Annual revisions will probably be necessary to keep pace with changing management demands and improvements in hatchery efficiency.

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More than 3,000 mature chum salmon from Asian and American waters and high-seas areas have been examined for parasites as part of the research program of the International North Pacific Fisheries' Commission. Thirty species of parasites have been identified and studied with special regard to their distribution patterns, but none has proved to be a reliable index of continental origin.

THE EFFECTS OF FISH REMOVAL AND OTHER FACTORS UPON REMAINING FISH POPULATIONS AT LAKE TRAFFORD, FLORIDA

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AN EXPERIMENTAL PROJECT OF FISH REMOVAL was begun at Lake Trafford, Collier County, Florida, on June 14, 1954, and terminated in February 1955. The purpose of the removal was to determine changes which might occur in rough-fish and game-fish populations during and after such removal. Financial support was obtained through the Federal Aid to Fish Restoration Program.

Preliminary studies showed Lake Trafford to be approximately 1,400 acres in size (determined by planimeter readings) and to have an average depth of 5.5 feet (July 28, 1955). The deepest area was 8.0 feet.

The bottom of the lake consisted primarily of soft mud and muck underlaid with hard-packed sand. Much of the bottom was covered with a layer of hyacinth debris--a result of a hyacinth-control operation conducted by the Game and Fresh Water Fish Commission and of years of the plant's presence in the lake (Luethy 1955). The pH readings ranged from 6.0 to 8.0 in 19 determinations. During high water the lake extended into the adjacent glades-type country. In the past the lake was connected, probably by means of canals, with salt water--as was indicated by the presence of tarpon (table 1).

Methods

The haul seine was operated by three crew personnel and one supervising biologist. Mesh sizes of the 855- to 1,000-yard nets used were changed during the period of study. Changes were necessitated by deterioration of webbing; they were made also to reduce the mesh size in an effort to increase average catch,

TABLE 1.--List of common and scientific names of the fishes taken by haul seine in Lake Trafford, June 14, 1954, through February 18, 1955.

Common name	Scientific name
Florida spotted gar	Lepisosteus platyrhincus De Kay
Bowfin	Amia calva Linnaeus
Tarpon	Tarpon atlanticus (Valenciennes)
Gizzard shad	Dorosoma cepedianum (LeSueur)
Chub sucker	Erimyzon sucetta (Lacepede)
Golden shiner	Notemigonus crysoleucas bosci (Cuvier and Valenciennes)
Yellow bullhead	Ictalurus natalis (LeSueur)
Speckled bullhead	Ictalurus nebulosus marmoratus (Holbrook)
Largemouth bass	Micropterus salmoides floridanus (LeSueur)
Stumpknocker	Lepomis punctatus punctatus (Valenciennes)
Bluegill	Lepomis macrochirus purpurescens Cope
Redear	Lepomis microlophus (Gunther)
Warmouth	Chaenobryttus coronarius (Bartram)
Black crappie	Pomoxis nigromaculatus (LeSueur)

and to determine the extent of smaller size groups of fish present.

A numerical increase of smaller fish followed a reduction of stretched mesh size. The increase amounted to 10 percent crappie, 1 percent bluegill, and 7 percent gizzard shad. As the changes in size composition of fish were abrupt after net modification, they were probably caused by those changes rather than by growth of smaller fish to a catchable size.

Fish Sampling Method

The method of obtaining samples for the total-weight-of-haul calculations was similar to that used in other haul-seine studies in Florida during which game fish were released. As fish were being bailed from the pocket of the net and sorted, "sample dip nets" were taken. Number and total weight of each species were obtained from the dip-net sample and recorded. Efforts were made to obtain samples of the dip nets during the entire bailing period. A count of dip nets taken from the pocket was obtained, and the total catch was based on the figures, i.e., average weight of the dip-net samples multiplied by total number of dip nets, plus weight of gar, mudfish, catfish, and others which were counted and weighed separately. Of the total number of dip nets, those used as samples ranged from 4.9 to 13.7 percent and averaged 9.5 percent on a 10-haul basis.

Data were grouped by 10-hauls because those sample groupings were more satisfactory for analysis of data than separate hauls or the hauls made during a month. There were large fluctuations of catch from haul to haul and a variation of 4 to 20 hauls per month. There were several reasons for greater coverage in one portion of the lake at times and less coverage at others. Chief of these reasons were strong winds and efforts made by crew members to return to areas where larger catches had been made. Also, some areas of the lake were difficult to seine because of soft mud or rock bottom.

The average haul encompassed an estimated 87.5 acres. The largest haul (during which some escape was believed to have occurred) was 150 acres in extent. A total of 10,678 acres, or 7.6 times the area of the lake, was estimated to have been covered during the 122 hauls.

Largemouth bass, black crappies, bluegills, redear, warmouth, and stumpknocker were sorted and released, except for a few which died or were retained for detailed examination. Gizzard shad, gar, bowfin, catfish, sucker, shiners, and others were killed and disposed of in the lake.

Results

During the study a calculated total of 230,753 pounds of fish was netted. Of these, 184,176 pounds (132 pounds per acre) were removed. The average catch of all fishes declined for the first 40 hauls: 3,945 to 796. During the remainder of the study a general increase to an average of 2,460 pounds occurred, with the exception of the ninth 10-haul sample, in which 1,483 pounds were recorded. Average catch did not consistently decline with the number of seine hauls completed. Also, there was not a consistent increase in average catch of the released species.

Changes in the average catch of the major carnivorous species (spotted gar) removed from the lake were much as the changes in major released carnivorous species (black crappie). A similar relationship existed between the major removed forage species (gizzard shad) and the major released forage species (bluegill). Of the two major released species (bluegill and black crappie), the bluegill contributed larger average poundages per haul at the end of the study than at the beginning. Bowfin and speckled bullheads, although minor portions of the removed poundages, also were present in larger poundages at the end of the study.

Comparison of average catch and water levels at the time of the 10-haul samples exhibited generally an inverse relationship, although the largest catches did not occur at the lowest water level. In the period of study, water levels ranged from 18.62 to 20.36 feet above mean sea level.

TABLE 2.--Haul seine catch in Lake Trafford, June 14, 1954, through February 18, 1955, on a ten-haul basis.

Ten-haul sample	Total pounds caught	Total pounds removed	Total pounds of shad	Numbers of acres covered	Average water level	Average temperature	Average pounds removed per acre
1	39,449	32,064	28,150	935	18.90	79.6	34.29
2	30,533	26,516	24,510	1,085	19.17	80.7	24.44
3	19,090	15,429	14,440	940	19.40	80.9	16.41
4	7,964	6,750	6,260	965	19.67	83.1	6.99
5	8,904	7,240	6,840	1,045	19.64	81.7	6.93
6	11,714	9,238	8,780	1,025	20.10	77.2	9.01
7	13,364	9,578	8,740	800	19.83	67.1	11.73
8	17,423	14,335	13,260	790	19.79	69.6	18.15
9	14,833	10,945	10,150	658	19.63	62.3	16.63
10	17,255	13,502	12,150	770	19.40	62.9	17.53
11	23,442	19,350	17,210	810	19.33	60.2	23.89
12	24,601	17,450	14,580	740	19.34	63.9	23.58

When gauge readings were highest, and catches lowest, the water level was considered very high. The subsequent increase in average catch of the 4th to 12th 10-haul samples was accompanied by a decreasing water level.

A relationship similar to that between average catch and water level occurred between average catch and average air temperature. Lowest temperatures were not accompanied by largest average hauls, but a decrease in temperature was followed by an increased catch. The relationship has been described for Florida waters in other studies (DeQuine, 1950, 1953; Huish 1954; Moody 1954).

Length-frequency studies, including over 17,000 shad and 9,000 crappie measurements, indicated that a progressive growth of shad to larger size classes occurred and may have been partly responsible for the increased weight from the 7th through the 12th samples of catches. Shad formed about 90 percent of removed poundages and 72 percent of total catch (tables 2 and 3).

TABLE 3.--Composition of the haul-seine catch in Lake Trafford, June 14, 1954, through February 18, 1955

/Estimated water area covered: 10,678 acres; number of hauls: 122/

Species	Pounds caught	Percent composition	Pounds per acre	Average pounds per haul
Largemouth bass	587	0.2	0.29	3.3
Black crappie	32,624	14.1	23.30	267.4
Bluegill	12,715	5.5	9.08	104.4
Shellcracker	837	.4	.60	6.9
Warmouth	3	-------	------	-------
Stumpknocker	-------	-------	------	-------
Speckled bullhead	902	.4	.64	7.4
Yellow bullhead	22	-------	.02	.2
Spotted gar	9,240	4.0	6.60	75.7
Mudfish	7,186	3.1	5.13	58.9
Gizzard shad	166,587	72.2	118.99	1,365.5
Chub sucker	6	-------	.00	-------
Golden shiner	47	-------	.03	.4
Tarpon	185	.1	.13	1.5
Fish				
Total-------	230,753	-------	157.40	1,891.5
Removed-----	184,176	79.8	131.56	1,509.7
Turtles				
Predatory---	90	-------	------	-------
Nonpredatory	718	-------	------	-------

Seine studies in Florida have shown that a high average percentage catch of shad was associated with poor sport-fishing success. This was also in evidence at Lake Trafford. At the time of these seine hauls, bass were found to constitute 0.2 percent of the catch. The bass taken averaged 2.6 pounds in weight and provided no sport fishing. The black crappie made up 14.1 percent of the fish population taken in the nets and was the major species sought by sport fishermen. However, very little sport-fishing effort was expended for any of the game species. Bluegill and redear formed 5.9 percent of the seine catch.

Summation of the total catch, including total pounds, percentage composition (by weight), average pounds per acre, average pounds per haul, and total pounds removed of species caught during the study, appears in table 3. As the haul seine was the major sampling device and catch results may be considered as an index to productivity of the lake, a comparison of average catch in Lake Trafford with average catch in other lakes with similar devices indicated that, although slightly lower, Lake Trafford was similar to other lakes in Florida.

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A fishway installed at the large hydroelectric dam near Pitlochry, in Scotland, has proved successful in carrying salmon safely past the dam during their migration up the River Tummel. Spent salmon, however, do not often use the fishway to return to salt water; at the end of the spawning season many fish can be seen leaping just above the dam. Visitors to the area have been told that only a very small percentage of these kelts can be expected to return to the river again if they do return to salt water.

Linings made of fibreglass cloth are being used on an experimental basis in the wooden troughs of a hundred-year-old commercial hatchery in southern Scotland.

OVARIAN FLUID: ITS ROLE IN FERTILIZATION

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FISH-CULTURISTS, in general, do not agree as to the time of actual fertilization of artificially spawned salmonid eggs. Some fish-culturists believe that the eggs are fertilized in the milt before water is added, while others are equally certain that fertilization is not achieved until after water is added. This difference of opinion has probably existed since the advent of the dry spawning method, in which milt is added to the eggs without the presence of water in the spawning pan. According to the "Manual of Fish-Culture" published by the U. S. Commission of Fish and Fisheries in 1900, it was known that eggs and milt from salmon maintain their viability for many hours if held in open containers, that both eggs and milt soon lose their viability when water is added, and that the milt and fluid around the eggs can be "utilized in impregnating masses of eggs when there is a scarcity of males, as sometimes occurs toward the close of the spawning season."

The object of the studies reported here was to confirm some of the foregoing observations and to determine whether salmonid eggs could be fertilized without the supportive action of water.

Materials and Methods

Mature silver salmon (Oncorhynchus kisutch) were obtained from the Washington State Department of Fisheries, and mature cutthroat trout (Salmo clarki clarki) were obtained from the Washington State Department of Game. All fish were killed, and the carcasses were wiped dry with absorbent towels. The milt was expressed directly into small, dry vials. The eggs were removed through an incision in the abdominal wall and collected in dry glass beakers. Care was taken prevent the rupture of eggs or the inclusion of liquids other than ovarian or coelomic fluids.

For each test, eggs from individual females or eggs from two or four females were placed in a beaker: this group of eggs was then divided into smaller lots in other beakers. Milt from one or two males was dispensed with a medicine dropper as needed. The eggs were killed and cleared in Stockard's solution[1/] after being incubated in water for 10 days at about 45° F. Fertility was easily determined at this stage because the fertilized eggs contained clearly discernible embryos.

Results and Discussion

Microscopic observation, which is sometimes used to determine salmonid

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During this work the junior authors were trainees at the In-service Training School at the Western Fish Disease Laboratory.

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Mr. Dickeson's present address: National Fish Hatchery, Leavenworth, Washington.

1/ Formalin, 5 percent; glacial acetic acid, 4 percent; glycerin, 6 percent; and water, 85 percent.

sperm motility, demonstrated that both water and ovarian fluid initiated tremendous sperm activity; but in each case the activity subsided in a few seconds. In order to obtain evidence that fertilization could occur in egg-milt mixtures before the addition of water, a solution that would wash off and kill all excess sperm without deleterious effects on the fertilized eggs was required. According to Dr. Frank G. Lowman[2/] of the University of Washington, Seattle, Washington, a normal solution of sodium chloride (5.8 percent salt) destroys the cell membrane of salmonid spermatozoa and neutralizes the surface charges of the nucleoprotein granules in the nuclei. Thus, the nucleoprotein granules become unfolded into long, thin molecules and are released into solution with the formation of an extremely viscous gel.

To make certain experimentally that the salt solution actually killed the excess spermatozoa, sperm were subjected to the salt solution and to water as a control before being subjected to groups of eggs. One experiment with cutthroat trout indicated that sperm suspended in water for 10, 20, and 30 seconds effected fertilization of 88, 59, and 3 percent in one case and 84, 70, and 0 percent in another. Sperm suspended in 5.8-percent salt solution apparently were destroyed, as no eggs were fertilized. Also, in this experiment with cutthroat trout, eggs of one lot which had been mixed with milt and washed in water before incubation were 100 percent fertile; whereas three lots that were washed with the 5.8-percent salt solution before being placed in water for incubation showed 75, 82, and 93 percent of the eggs to be fertile.

[2/] Private communication.

A somewhat similar set of experiments was conducted with silver salmon. One set of a triplicate series of fertilized eggs was washed in water and another with the 5.8-percent salt solution to kill all sperm which had not entered the eggs. The eggs and sperm were held in contact for 10 minutes before being washed. Those that were washed in water had a fertility of 86, 99, and 100 percent; whereas those that were washed with the saline had a fertility of 76, 99, and 98 percent. The gel phenomenon was observed when fertilized eggs were washed with the 5.8 percent salt solution. This indicated that the eggs were fertilized in the ovarian fluid before the addition of water, because the salt solution destroyed the sperm which had not entered the eggs.

In an effort to determine further the effect of ovarian fluid on sperm, fresh eggs were fertilized with milt which had been held in contact with eggs for different periods of time. Three experiments using silver salmon eggs showed that milt held with eggs and the accompanying ovarian fluid for 1 hour would still fertilize 74, 85, and 95 percent of groups subsequently exposed to this milt.

It appears that ovarian fluid not only activates sperm but may prolong its viability. Other results may possibly be obtained by varying experimental conditions, such as altering the ratio of ovarian fluid to milt.

Summary

This study presents evidence that sperm from cutthroat trout and silver salmon were activated by ovarian fluid, that the ovarian fluid prolonged the viability of the sperm, and that eggs can be fertilized in ovarian fluid without the addition of water.

In three lakes in central Sweden, rotenone applied in concentrations of 0.5 to 0.6 p.p.m. during the summer and fall of 1958 produced total kills among most of the zooplankton, many of the epiphytic and bottom animals, and some of the phytoplankters.

SUITABILITY OF WELL WATER WITH A HIGH IRON CONTENT FOR WARM-WATER FISH CULTURE

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DISSOLVED IRON, principally in the form of ferrous bicarbonate $Fe(HCO_3)_2$, is a common component of well water. In fact, high iron content is characteristic of some of our most abundant ground water supplies. It is prevalent in at least part of the great American aquifer of the Mississippi River bottoms starting at St. Louis and extending to New Orleans.

When pumped from a well, the iron-containing water may be crystal clear; but when the water is exposed to air and permitted to stand, it turns brown because of the formation of a hydrated oxide ($Fe_2O_3.XH_2O$). Also, the water stains containers or any object that it wets. A quick test for the presence of the iron may be made by heating the sample. If the iron is present, the water will turn brown.

Schaeperclaus made the following statements (1933: 58) concerning iron in spring water:

> ... the presence of iron denotes a poorly productive water .... As soon as the water becomes alkaline and contains sufficient oxygen, the iron is precipitated as a red iron hydroxide mud. One can therefore get rid of it through airing of the water and introduction of chalk.
>
> . . . . .
>
> Iron is very easily deposited upon the alkaline gills of fishes and also upon the eggs of trout, causing irritation and blocking of the respiratory channels. In other words, it becomes occasionally quite noxious. It causes necrotic white spots upon the gills of trout fry. Hence, it is important that waters feeding trout ponds and hatcheries be kept free--or freed--from larger deposits of iron.

Ellis, Westfall, and Ellis made the following statement (1948: 71):

> ... The presence of more than 1 or 2 p.p.m. of iron usually points to polluted water or to conditions which may be unfavorable to fishes.

A person reading the above statements and seeing, for the first time, water containing the suspended hydrated iron oxide would be led to believe that the water is rather toxic to fish. My experience is to the contrary.

The Fountain Bluff Hatchery (a small private bait hatchery) at Gorham, Illinois, is supplied by a well that produces water containing 35 p.p.m. total iron (gravimetric and colorimetric), which results in the formation of 214 p.p.m. turbidity produced by ferric hydroxide (measured by Hellige Turbidimeter). This turbidity is such that when the water is pumped into an empty pond, it will become extremely turbid with hydrated ferrous oxide. In 2 to 3 days after pumping has ceased, the flocculent material will settle out and the pond will become clear. If the water is pumped into a pond that already contains water, the incoming pump water will immediately turn brown.

The important point, as far as the fish-culturist is concerned, is whether the water can be used for fish-cultural purposes. On the basis of the following observations, I am of the opinion that the water is quite satisfactory for most warm-water hatchery uses:

1. When ponds were filled with water of high iron content and stocked immediately with fish, and the water level was thereafter maintained by use of the water, no evidence of iron toxicity could be noted. The fish prospered and reproduced.

2. When water was pumped into ponds containing thousands of newly hatched golden shiners (Notemigonus crysoleucas) and into other ponds containing thousands of newly hatched fathead minnows (Pimephales promelas), no mortality occurred and the young fish appeared healthy in every respect.

3. Fifty 3-inch golden shiners were placed in a holding tank that was 3 feet wide and 24 feet long and had a water depth of 8 inches. After the fish had been tempered to the well-water temperature, a flow of 20 gallons per minute directly from the well was allowed to run into the tank and then out by way of an overflow. At the end of 3 days, the shiners were in excellent condition.

There is, however, at least one disadvantage to the use of water of high iron content. It cannot be recommended for cold-water hatcheries. When so used, the excess turbidity makes the water undesirable, and it is hardly possible to oxidize the iron and permit it to precipitate without having the water become too warm. For this same reason, iron-containing water is not satisfactory for holding warm-water fish at low temperatures.

Acknowledgments

The Illinois Department of Conservation cooperated in this study.

The writer wishes to thank Mr. George Crouse for making the iron determinations.

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SYMPOSIUM ON COLLECTION AND UTILIZATION OF WATER-QUALITY DATA

The Public Health Service announces a symposium on the collection and utilization of water-quality data, to be held at the Sanitary Engineering Center at Cincinnati, Ohio, on August 23-25, 1960.

The symposium will bring together representatives of organizations currently engaged or interested in the establishment, instrumentation, and operation of water-sampling networks and other related activities in water resources management. It will provide an opportunity to exchange information on objectives, organization, and operation of such networks; on the analysis and utilization of data; and on existing needs in the field of water-quality measurement. Particular emphasis will be placed on automatic instrumentation.

When ready for distribution, the program will be sent to organizations and individuals known to be interested in this field. Anyone who wishes to receive the agenda and other announcements may write to: The Director, Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio.

NOTES ON FIRST-YEAR GROWTH OF SEVERAL SPECIES OF MINNESOTA FISH

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IN A STUDY OF AVAILABLE FISH FOOD in Linwood Lake, in southeastern Minnesota, seine hauls were made on 15 dates during the summer of 1954 in order to sample forage-size fishes.[1] As the period, May to mid-October, covered the spawning seasons for most of the species present, information about first-year growth of a number of the species commonly caught in the seine was obtained by computing mean lengths on each seining date. The data include information about growth of the spottail shiner (Notropis hudsonius), bluntnose minnow (Hyborhynchus notatus), Johnny darter (Boleosoma nigrum), banded killifish (Fundulus diaphanus), pike (Esox lucius), largemouth bass (Micropterus salmoides), bluegill (Lepomis macrochirus), pumpkinseed (L. gibbosus), and the yellow perch (Perca flavescens).

Methods

Fish collections were made with a 30-foot seine (1/4-inch mesh) with the center portion lined with 5/32-inch netting to retain post-larval fish. Seining was done at four stations along the lake shore, and samples from all the stations for each seining date were combined. Mean total length in millimeters of each of the species was determined for each date. Where the seine samples were large and the variability of fish size was small, random subsamples were measured.

Mean lengths of age-group 0 minnows, darters, killifish, pike, bass, and sunfish are shown in table 1.

Yellow perch older than age-group 0 were commonly caught. One-year-old fish were easily separated from those of other ages on the basis of length or, where doubtful, by examination of scales.

Mean lengths of perch of age-groups 0 and I are shown in table 2.

Discussion

Growth rates follow smooth curves for species where considerable numbers of fish were regularly obtained. Growth was most rapid from mid-June to mid-August. By September the growth had decreased markedly. No consistent increase in the length was shown by any of the species after the seining on September 17.

Young-of-the-year perch caught late in September averaged slightly more than 70 millimeters in length, whereas perch

[1] Lux, Fred E. An investigation of the causes of seasonal changes in angler success in Linwood Lake, Minnesota. Master's thesis. Unpublished, 88 pp. University of Minnesota. 1958.

Mr. Lux is now with the Bureau of Commercial Fisheries, Woods Hole, Mass.

TABLE 1.--Mean total lengths, in millimeters, of 0-group spottail shiners, bluntnose minnows, Johnny darters, killifish, pike, bass, bluegills, and pumpkinseeds from Linwood Lake during the 1954 season, and numbers of fish in each sample used to obtain mean length

(N - Number; L - Length)

Date	Spottail shiner		Bluntnose minnow		Johnny darter		Killi-fish		Pike		Bass		Blue-gill		Pumpkin-seed[1]	
	N	L	N	L	N	L	N	L	N	L	N	L	N	L	N	L
June 12	--	--	--	--	--	--	--	--	1	61	--	--	--	--	Same	
18	54	13	--	--	4	17	--	--	-	---	--	--	--	--	as	
29	28	21	4	15	14	23	--	--	1	109	37	36	9	11	for	
July 9	46	28	19	18	37	27	5	15	2	98	56	39	38	14	blue-	
15	20	31	14	18	34	31	10	22	3	138	73	44	76	16	gill	
27	1	38	49	26	38	35	21	28	-	---	64	59	77	22	thru	
Aug. 14	--	--	58	32	37	39	79	39	1	217	23	52	82	30	August	
28	3	54	58	38	9	39	22	44	2	254	11	62	77	33		
Sept. 7	--	--	62	36	48	40	77	44	-	---	12	65	85	36	53	43
17	5	63	21	47	25	47	34	47	-	---	7	77	67	38	41	44
24	--	--	38	53	21	47	13	50	2	279	19	91	78	37	44	44
Oct. 5	7	63	11	40	29	48	18	50	1	293	8	71	79	37	12	42
17	5	63	1	51	6	47	2	44	3	282	9	90	54	39	11	42

[1] Prior to September, 0-group bluegills and pumpkinseeds were combined because these small fish could not be readily distinguished by species.

of age-group I caught during the spring (May 5) were just under 70 millimeters in length. A difference in the goodness of growth during their first year possibly is responsible for this small difference in the length recorded for the fall-caught 0-group perch and the spring-caught I-group fish. Apparently Linwood perch of these age groups add most of their growth between May and mid-September. The growing season for other fish in Linwood Lake probably covers a similar part of the year.

The surface water temperature of Linwood Lake during the seining period ranged from 62° late in May to 78° F. in July and August. By mid-September the water temperature had again dropped to about 62° F.

TABLE 2.-- Mean total lengths, in millimeters, of yellow perch of age-groups 0 and I from Linwood Lake during the 1954 season, and numbers of fish in each sample used to obtain mean length

Date	Age-group 0		Age-group I	
	Number	Length	Number	Length
May 8	--	--	6	69
22	--	--	49	72
June 12	2	18	6	87
18	19	25	60	90
29	86	35	70	93
July 9	83	43	59	98
15	62	46	23	95
27	60	50	38	94
Aug. 14	60	54	11	98
28	35	57	27	103
Sept. 7	51	59	53	103
17	87	60	6	107
24	93	62	63	106
Oct. 5	41	61	21	110
17	43	63	5	118

Inshore coastal waters can be cultivated to produce far more than the richest farmland.

CHOICE BETWEEN TWO SIZES OF FORAGE FISH BY LARGEMOUTH BASS UNDER AQUARIUM CONDITIONS

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Southern Illinois University
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A LARGE PART OF WARM-WATER FISH MANAGEMENT involves the establishment of a balance between forage fishes and predaceous fishes. The most important warm-water predator is the largemouth bass (Micropterus salmoides), and a more complete knowledge of its feeding habits will be helpful in understanding how to maintain balance. The effect of such things as palatability, vulnerability, and size of forage fishes on the feeding behavior of the largemouth bass has not been thoroughly investigated. This study is an attempt to obtain better understanding of the problem of size preference.

The method of study consisted of holding four largemouth bass in circular stock-watering tanks and offering these bass green sunfish (Lepomis cyanellus) of two size classes. The tanks were equipped with a central drain, and fresh water flowed into them at the rate of 15 gallons an hour. The water was maintained at a temperature of 70° to 76° F. during the study.

The largemouth bass used had been obtained 4 to 10 months before the beginning of the study. While being held, they were fed green sunfish at the rate of 1 percent of their body weight per day. The weight and condition index of each largemouth bass when it was first obtained was as follows: Bass No. 1--681 grams, 2.65; No. 2--630 grams, 3.20; No. 3--284 grams, 2.56; No. 4--296 grams, 2.17. In order to cause as little disturbance of their feeding behavior as possible, the largemouth bass were not weighed again until the end of the study.

At the beginning of the study five green sunfish with an average weight of 3 grams and five with an average weight of 11 grams were made available to each largemouth bass. Each day the number of sunfish that had been eaten or had died was noted and fish of the same sizes were added. The weights of the large sunfish ranged from 7.0 to 16.5 grams; weights of the small ones ranged from 1.9 to 4.0 grams. The study extended over a period of 48 days.

To determine whether the large green sunfish utilized the smaller ones as food, five of each size were held in a 15-gallon aquarium. The aquarium was equipped with an overflow pipe, and water from the same source as that used in the experimental tanks entered the aquarium at the rate of 4 gallons an hour. Each day the number of sunfish that had died or had been eaten was recorded and new fish of the same sizes were added.

Results

The large green sunfish constituted 88 percent of the total number of green sunfish eaten by the largest bass, 45 percent of the total number eaten by the

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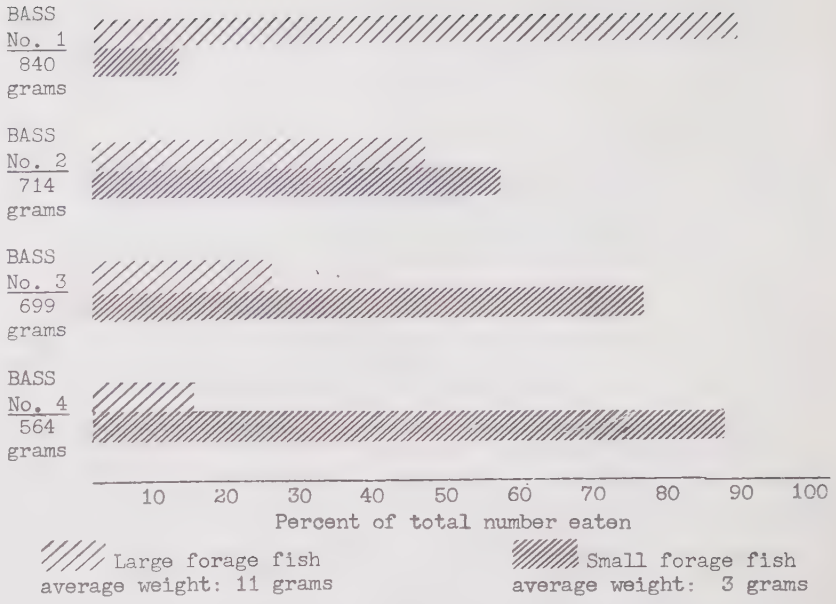
This paper is based on a master's thesis submitted to the graduate faculty of the Department of Zoology, Southern Illinois University.

second-largest bass, 25 percent of the total number eaten by the third-largest bass, and 14 percent of the total number eaten by the smallest bass (see figure). The coefficient of correlation between the size of largemouth bass and the proportional number of large fish eaten by each bass was 0.93. The number of small green sunfish consumed by the larger sunfish was negligible.


BASS No. 1 840 grams
BASS No. 2 714 grams
BASS No. 3 699 grams
BASS No. 4 564 grams
10 20 30 40 50 60 70 80 90 100
Percent of total number eaten
Large forage fish average weight: 11 grams
Small forage fish average weight: 3 grams

Size preference of largemouth bass during a period of 48 days.

The weight and condition index of each largemouth bass at the end of the study was as follows: Bass No. 1--840 grams, 2.85; No. 2--714 grams, 2.78; No. 3--699 grams, 3.25; No. 4--564 grams, 2.77. All the largemouth bass increased in weight from the time of capture until the end of the study, indicating that they were feeding adequately; all except bass No. 2 showed improvement in condition index.

The data indicate that a particular size of largemouth bass may show a preference for a particular size of forage fish. Even with a small variation in size of bass, there was a difference in preference, as the larger bass preferred larger forage fish and the smaller bass preferred smaller forage fish (see figure).

Possibly the relative sizes of forage and predator fishes should be given more consideration when the question of population balance is being considered.

Acknowledgments

The Cooperative Fishery Research Laboratory placed various facilities at the writer's disposal. The work was done under the direction of Dr. W. M. Lewis.

Before 1941, when a dam was built on Lake Minnewanka, in Banff National Park, the major sport fish was the lake trout (Salvelinus namaycush), which used an introduced species of cisco and the native Rocky Mountain whitefish as food. Since the completion of the dam, the value of this sport fishery has declined markedly. The weight of the lake trout taken from Lake Minnewanka now is rarely greater than one pound. At this weight, normally, the trout ceases to feed on plankton and bottom organisms and starts to feed on fish. Now, however, this change in the diet is not made and the growth of the trout is arrested. Apparently the fluctuations in the water level of the lake have caused an ecological separation of the lake trout and the forage fish.

TECHNIQUES FOR STUDYING HERRING SCALES AND OTOLITHS

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and
Bernard E. Skud, Bureau of Commercial Fisheries, Galveston, Texas

THIS PAPER describes (1) a technique for mounting and photographing Atlantic herring (Clupea harengus) scales and (2) a technique for clearing and photographing herring otoliths. Scale-mounting techniques may be divided into three general categories: mounting dry on microscope slides, mounting in transparent media on slides, and making impressions in plastic. Otoliths may be cleared in xylene and mounted permanently in a medium or studied without further treatment. The selection of one of these techniques depends on the structure of the scales or otoliths, the facilities available, the information desired, and the preference of the individual.

Personnel at the Boothbay Harbor Biological Laboratory mounted scales in the manner described below. To free them of mucus and foreign matter, the scales were soaked in a watch glass containing a mixture of fresh water and detergent. Very dry scales required several hours' soaking to clean them properly. After soaking, a scale was removed from the watch glass, held on the posterior edge with forceps, and rubbed gently between the thumb and forefinger. The scale was then dipped in clean water and blotted on a paper towel. The clean scale was immersed in a transparent mounting medium, then touched lightly to a paper towel to remove excess liquid, and placed on the microscope slide. A folded paper towel was laid over the scale and pressed with several forward and backward motions of a printer's roller to fix the scale to the slide. If the scale curled on drying, additional medium was placed on the scale, which was again rolled. Fresh scales seldom curled, and they proved to be the most satisfactory to mount; however, scales that had been dry for several years were mounted without much difficulty. The mounting medium consisted of a mixture of 1 part liquid glue (Stafford's Clear Mucilage) to 4 or 5 parts water; a stronger solution was required if the scales were old or brittle.

To obtain photographic reproductions of scales mounted in this manner, we made a fiberboard negative holder to hold the microscope slides in the enlarger. This holder masked all of the light except that which passed through the scale being reproduced. The scale image was brought into sharp focus on a sheet of white paper placed on the easel. The enlarger light was then turned off, and the white paper was replaced with photographic paper and exposed. Exposure time and f:settings varied in accordance with the magnification desired and the scale structure. For magnification of 30X with scales from 2- and 3-year-old fish, we used Kodabromide F-5 paper with a 150-watt enlarger bulb and a 3-second exposure at f:22. We treated the scales as low-contrast negatives and took care to prevent their overexposure. Developing followed standard techniques, except that a short-stop solution was unnecessary because of the brief exposure time (2 to 10 seconds). Figure 1 shows an Atlantic herring scale printed from the technique described.

Because racial and growth studies of herring depend on examination of otoliths

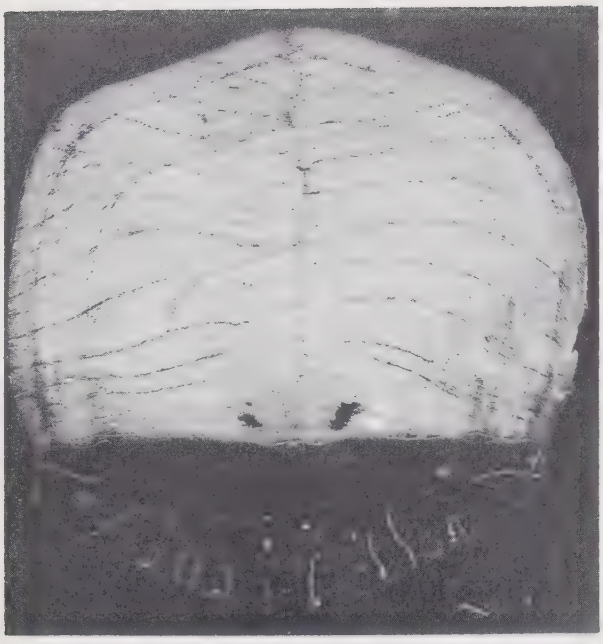

FIGURE 1.--A print of a scale from an immature Atlantic herring.

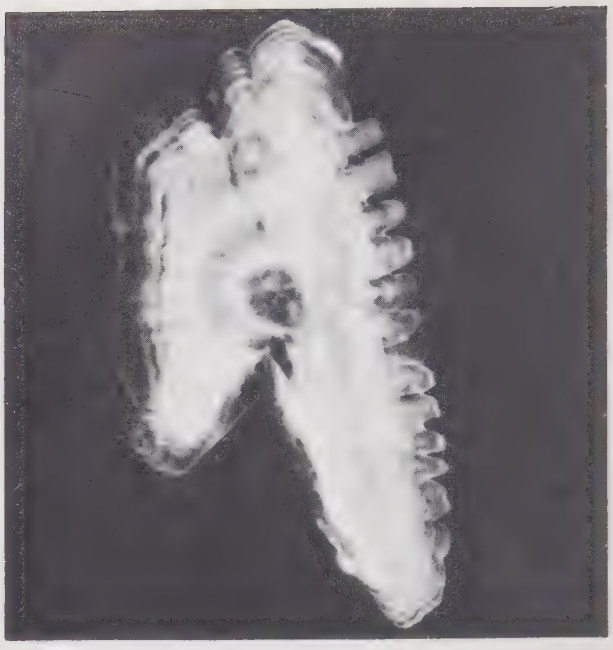

FIGURE 2.--A print of an otolith from a mature Atlantic herring.

as well as scales, it is desirable to have clear photographs for the comparison of various otolith features such as shape, size, general texture, nuclear characteristics, and size and spacing of the growth zones. Detection of these differences can assist in the identification of individuals from various spawning groups.

We used the following technique for clearing and photographing otoliths. The otoliths were cleared in xylene for 4 to 5 minutes and then placed on a microscope slide without any mounting medium. The slide was placed in the enlarger in the same manner as the mounted scales. The otolith's uneven surface made focusing difficult. Size of the otolith must be taken into consideration in the determination of exposure time and f : settings. For young herring otoliths magnified 50X, a 300-watt enlarger bulb with a 30-second exposure at f : 8 proved satisfactory. We used the same setting for all otoliths from the same size group. As in photographic reproduction of scales, a strong light source is a necessity, and a high-contrast paper must be used. With the resultant satisfactory prints, we were able to make age determinations in agreement with scale readings. Figure 2 shows a print of an otolith from a mature herring.

By using the methods of handling and mounting scales and otoliths described in this paper, clear large prints may be obtained directly with a photographic enlarger. This provides a rapid, efficient, and inexpensive procedure for the examination of scales and otoliths for racial as well as age and growth studies.

Fish in the St. Lawrence River have a better supply of live food because of the quantity of guano dropped by the Quebec flock of snow geese (estimated at 100,000 in 1957) during the annual 5-month sojourn in that area.

INEXPENSIVE SELF-CLEANING INVERSE AQUARIUM FILTER

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THE EXPERIMENTAL BIOLOGIST, AQUARIST, HATCHERY ATTENDANT, AND STUDENT, when trying to maintain aquaria or troughs free of silt, debris, or decaying matter, are plagued with the troublesome task of tearing down, cleaning, and rebuilding filters. Problems arise because ordinary filters, which operate on the gravity principle, deposit the unwanted material on top of glass wool, charcoal, or sand and soon become clogged and unsightly. These accumulations must be removed, and this operation is costly and time-consuming.

With a reversal of the filtering method, passing water up instead of down through the filtrant, costly waste of time and effort can be eliminated. Likewise, employing this principle and the modification cited here, which permits automatic self-cleaning to proceed, lessens filter maintenance and cost. Periodic flushing for cleaning purposes is simple and easy. The filter described here will adequately handle a 40-gallon aquarium with little maintenance other than occasional flushing of the settling chamber and/or filter material.

An inexpensive inverse filter (figure 1: A) can be constructed easily from a variety of materials, such as 1/8-inch Plexiglas or 1/4-inch plywood or boards, depending on the volume and size desired. This filter is a rectangle 12 inches long (overall), 6-1/4 inches wide, and 5-1/2 inches high. A center filter compartment (inside measurements, 3-7/8 by 6 by 9-3/8 inches) is constructed of two pieces of material, C and D (figure 1: A, B), to yield a filter volume of slightly more than 3,000 cubic centimeters. Panel C is a solid piece of material; while D is a piece in which 759 1/8-inch holes, 1/4 inch apart, have been made. The number of holes may be decreased or increased as the volume or use increases. The entire filter, as shown (minus filter material), will hold about 1-1/8 gallons (4,258 cubic centimeters) of water if filled to within 1/2 inch of the top. Depending somewhat on the amount and type of filter material used in compartment Z, the operating filter has a capacity of about 0.324 gallon (1,226 cubic centimeters). Note that the floor (E) of the filter is slanted at a 7° angle (figure 1: A, B). This refinement is necessary to assist the heavy non-filterable material to concentrate at one end of the filter. Water passing downward in compartment X carries the heavier matter down the incline toward the flushout opening (O). This process is aided by the swift current produced as water passes the small gap at Y. As the lighter unwanted material accumulates on the under surface of D, it, too, falls off and passes down the incline toward O. The results are longer life of the filter material and less costly operation.

Construction

Lay out pieces to be cut as in figure 2. Form a square by fastening panels A, A', B, and B' together with a Plexiglas suspension or Weldwood Contact Cement. A small aluminum corner clamp and miter vise will aid in this construction. Set

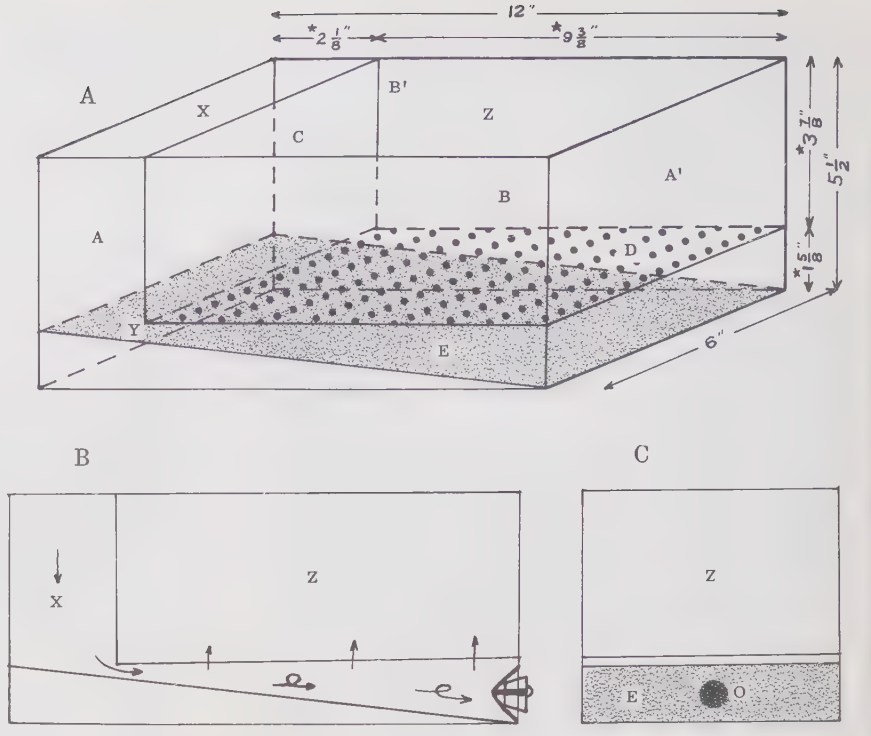

FIGURE 1.--

A.--Three dimensional view of inverse aquarium filter, illustrating inflow compartment X and filter compartment Z above the slanted floor E.

B.--Side view of filter. Arrows indicate direction of water currents.

C.--End view of filter, looking up the incline of E and through compartment Z toward compartment X. Y is the small space below Z.

* Inside dimensions.

panel C in place. Drill panel D, and insert it to fit snugly between C and A'. Attach panel E. As E is set at an angle, beveling the ends with sandpaper, file, or plane will be necessary to make a clean, smooth fit. Recoat all joints with Weldwood cement or a suitable substitute. If plywood or wood is used, the Weldwood is also usable for seams; but asphaltum or a suitable substitute should be applied before each piece is attached or inserted. Nail all pieces into place with lath nails or 1/2-inch brads, and coat the inner surfaces if of plywood or wood construction. Drill a 1-5/16-inch hole in the lower end of panel A' immediately below panel D and insert a No. 7 rubber stopper outfitted with a molly anchor screw. The wings of the molly screw should project from the smaller end of the rubber stopper.

Construction materials.--For 1/8-inch Plexiglas or 1/4-inch plywood:

A, A' = ends, 5-1/2 by 6 inches
B, B' = sides, 5-1/2 by 12 inches
C = panel, 4 by 6 inches
D = filter bed, 6 by 9-1/2 inches, drilled with 33 rows of 23 1/8-inch holes, 1/4 inch on square
E = floor, 6 by 11-13/16 inches

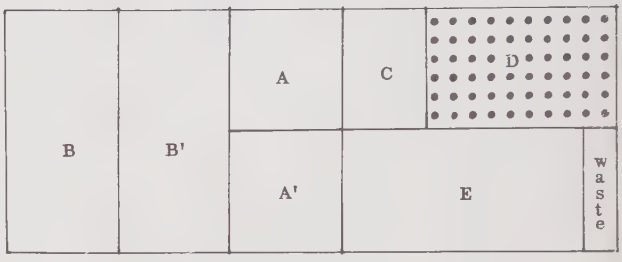

FIGURE 2.--Layout plan for various panels necessary to construct inverse aquarium filter from one piece of 12- by 30-inch material.

Comparison of Costs (Excluding Labor)

Plexiglas, 1/8-inch, 1 piece, 12 by 30 inches	$5.00	----
OR		
Plywood, 1/4-inch, 1 piece, 12 by 36 inches	----	$0.45
AND		
1 Bottle Weldwood	.25	.25
1 Molly screw	.15	.15
1 No. 7 rubber stopper	.09	.09
Nails or brads	----	.05
Tar	----	.25
	$5.49	$1.24

Commercially produced filters of comparable size, but not design, will cost between $12 and $25.

Suggested Modifications

Either a side or an end spillway lip can be attached to compartment Z. This would eliminate the necessity for an outlet siphon tube.

The flushout opening (O) can be modified in a number of ways depending on the size of the filter.

With increased size, a horizontal sliding-door arrangement can be devised such that a broom or scrub-brush can be inserted to clean off E or D.

A spigot can be utilized at O.

Attaching strips or pegs beneath D and adjacent to C would permit removal of C and D.

A separate, completely removable compartment can be made to slip into Z, thereby preventing any delay in the cleaning of the filter. This would be most useful in hatcheries where time is at a premium. The entire filtrant could be disposed of easily or cleaned as time permitted.

Sundry other possibilities are available and should be used, depending on size. Comparable volumes can be obtained if the height is increased instead of the width. This modification will be very effective if the filter has been made to hang on the side of an aquarium. For closed-system filtering of larger aquaria, a 2-inch increase in width for each 20 gallons of water is recommended; thus a filter that is 12 inches square and 6 inches high should adequately handle a 100-gallon aquarium.

Various uses can be ascribed to this filter. Compartment Z, minus filtrants, can be used by aquarists or, if large enough, by hatcherymen to hold spawning fish. Increased size of holes in D would permit eggs and newly born or hatched young to escape into the chamber below. Hatcherymen might use the larger filters in place of hatchery jars or to sort out different sizes of fingerlings.

AERATOR FROM A TWO-STAGE FUEL PUMP USED IN TRANSPORTING LIVE FISH

When live fish are to be transported, the equipment for the task should include an aerator from a two-stage fuel pump. If the vehicle in which the fish are to be transported requires only a one-stage fuel pump, this can be replaced with a two-stage unit. As soon as the two-stage unit has been installed, the intake side of the vacuum stage is fitted with a rubber hose and screen filter, and the pressure-outlet side is fitted with a hose that will carry the air to the fish tanks. If the fish-distribution car is already equipped with a two-stage fuel pump, the connections to the manifold and the windshield wipers can be closed and the vacuum stage can be fitted as described.

While the fish remain in the tanks, the fuel pump--and the car motor--must be kept running at all times. The air hoses and stones may be connected as they are needed.

The aerator from a two-stage fuel pump is a continuous, reliable source of air for the fish tanks. The total cost of its installation ($10 or less) is very small when compared with that of other motor-compressor units. The life of the unit is comparable to that of any standard piece of car equipment (any fuel pump) and can be expected to exceed by far the life of an accessory compressor.

--ROBERT O. ELLIS, Illinois Natural History Survey, Urbana.

A SMALL DRAG NET FOR CAPTURE OF BOTTOM FISH AND INVERTEBRATES

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Vancouver 8, British Columbia, Canada

DURING A STUDY ON COMPETITION IN MARINE FISHES, certain inadequacies were discovered in collecting techniques that involved the use of hand beach-seines and scoop nets. A small drag net that was designed during this study overcame some of these difficulties and allowed collection of very small fish at a known depth.

FIGURE 1.--Frame of the net.

Description of the Frame

The frame (figure 1) of the net is made of 0.75-inch solid round brass rod. The beam (figure 2: A) of the frame is 4 feet long; this is screwed and bolted at the ends to two runners (figure 2: B) and can be detached when the net is not in operation. A loop is welded at the center of the beam to hold one of the strings of the bridle. The runner has a 2-foot base (figure 2: C), which has a slight inward bend at the anterior arm. The anterior arm (figure 2: D) of the runner is 1 foot

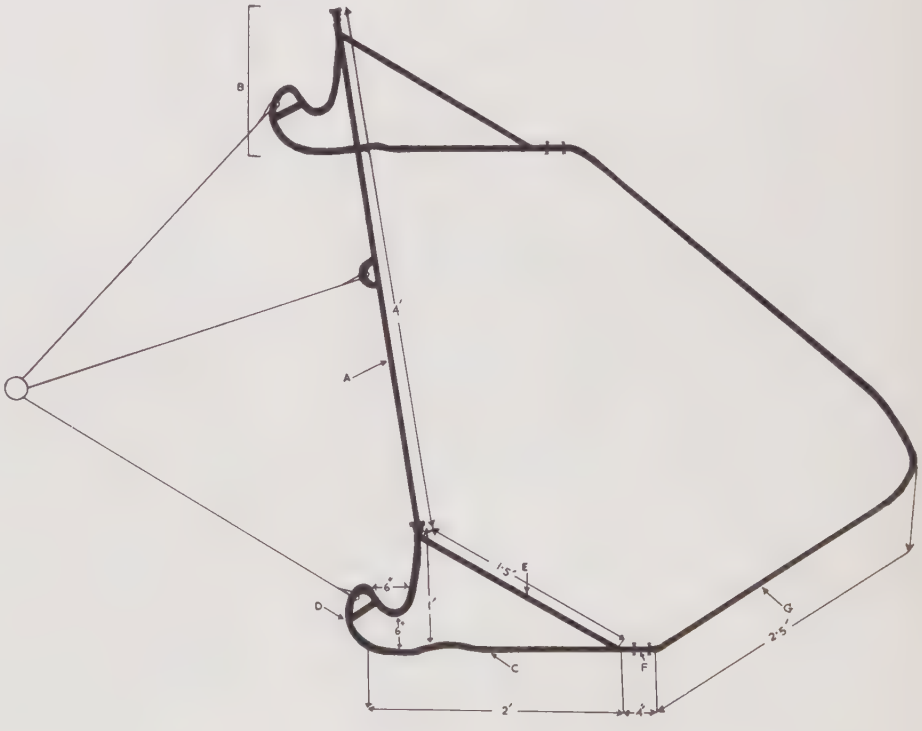

FIGURE 2.--Frame of the net.

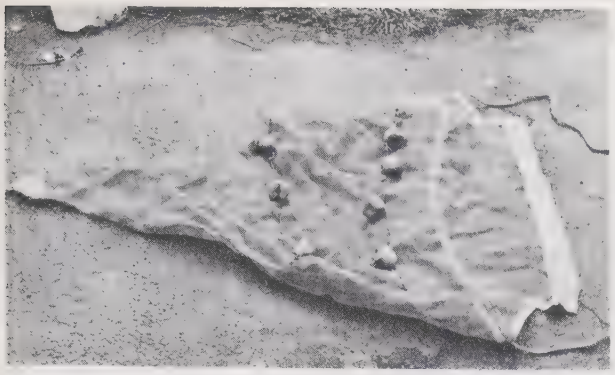

FIGURE 3.--The cone-shaped bag.

high and has a deep inward curve. A diagonal support (figure 2: E) runs between the front arm and the base of the runner. The latter two arms are made of one piece of rod. Welded to the basal arm is a hollow tube of brass (figure 2: F), about 4 inches long. This piece has two holes in it. This is where the trailing part of the runner (figure 2: G) is screwed and bolted to the proper runners. The trailing part is 2.5 feet long and curvilinear. The other two strings of the bridle are tied to the loops welded to the anterior arms of the runners.

Description of the Net

The cone-shaped bag (figure 3) is made of green plastic fly-screen, 18 mesh to an inch. The upper half of the bag is 6.5 feet long; the lower half, 5.5 feet long. The halves are stitched together with nylon twine. The mouth of the bag has a spread of about 4 feet and a 4-inch canvas border. The beam of the frame passes through the folded canvas in the upper half. The lead line is stitched to the canvas of the lower half of the bag, and its ends are tied to the loops in the anterior arms of the runners. The cod end, about 8 inches long, is also made of canvas. The opening at the posterior end is 6 inches wide; it can be closed or opened at will.

The resistance of the webbing to the water probably produces all the lifting effect necessary, but a few (seven) plastic floats are stitched to the upper half of the bag to help keep its mouth open. Round metal weights on the footrope result in an elliptical opening of the mouth as the net is dragged.

Operation of the Net

The net should be dropped slowly and horizontally from a boat to the water surface; only then should more rope be released. This way the net settles perfectly on the bottom. The net is dragged from an outboard motorboat (10 to 25 horsepower) at 2 to 2.5 miles an hour. An electric depth-sounder is also used from the boat.

Besides cottids, the net took flatfish, sand-lance, some very young and small unidentified fish, and a large quantity of mud shrimp.

The net can be used effectively on sand and sand-mud flats in shallow or deep intertidal waters. The maximum depth tried was 20 feet, but it seems likely that the net will be effective in deeper waters.

Advantages and Disadvantages

The frame is very light and can be disjointed, and is therefore easy to carry on field trips.

The net can be managed easily by two persons working from a boat. It can be operated by one person if not working from a boat or in deep water.

Bags of different mesh sizes can be placed on the same frame, depending on the size of the fish desired.

The net may be used as a standard measure for comparing catch sizes, in different areas or at different depths, if a record is kept of the speed of the boat and the time and distance the net is dragged.

The cost could be reduced considerably by using iron rod instead of brass.

Because of accumulation of sand and mud, water passes slowly through the bag.

Acknowledgments

Dr. P. A. Larkin and Dr. C. C. Lindsey offered suggestions and reviewed the manuscript. Dr. E. J. Crossman and D. E. McAllister helped to try out the net. N. Pelkey of the Locarno Boat Rentals also was helpful.

THE THERMOS BOX FOR FISH-CULTURISTS WITH ICE PROBLEMS

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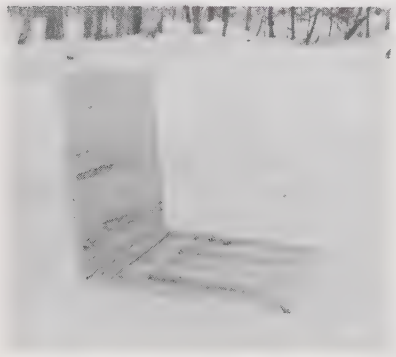
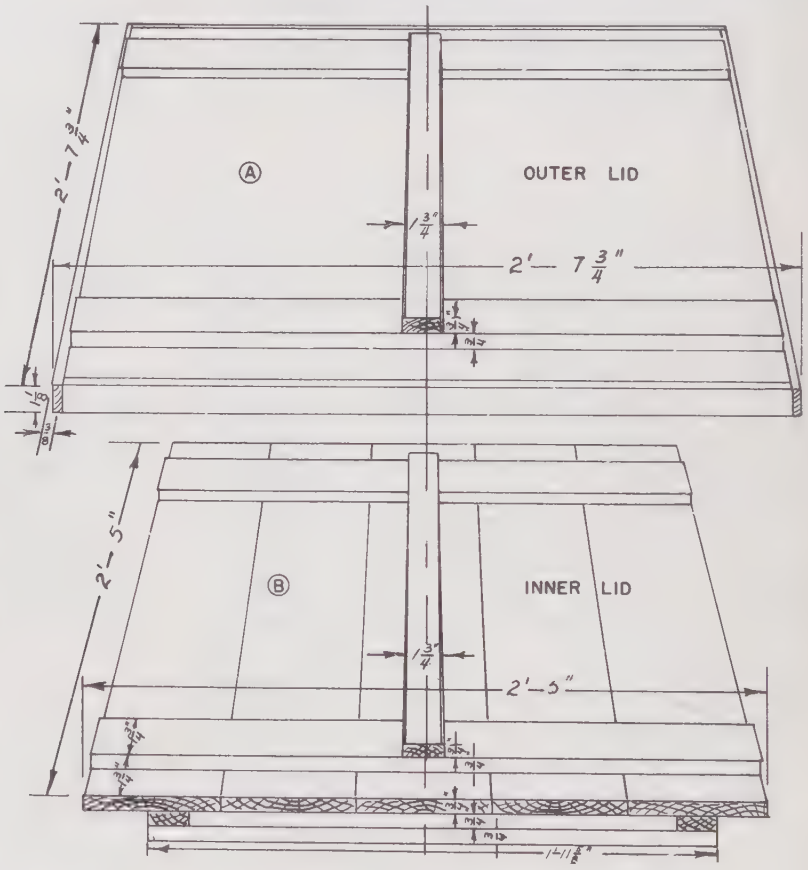
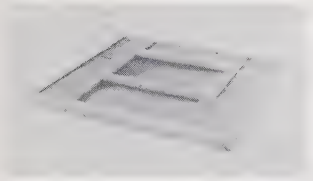
AN EXCEPTIONALLY COLD WINTER created thick ice on trout rearing ponds at the Thunder River Hatchery. Holes were required for feeding the fish and cleaning the screens in newly constructed dirt-bottom ponds. Openings in the ice were also needed for observing the condition and activity of the fish and for keeping a record of the mortalities. Heavy ice formed in the holes every day, and they had to be chopped out with an ice chisel. This was quite a chore, as 28 holes were required.

To cope with this problem, an insulated box that can be placed above the holes to prevent them from freezing over has been devised. Air spaces between the inner and outer covers and below the shelf in the lower part of the box act as insulators against the cold. The box is



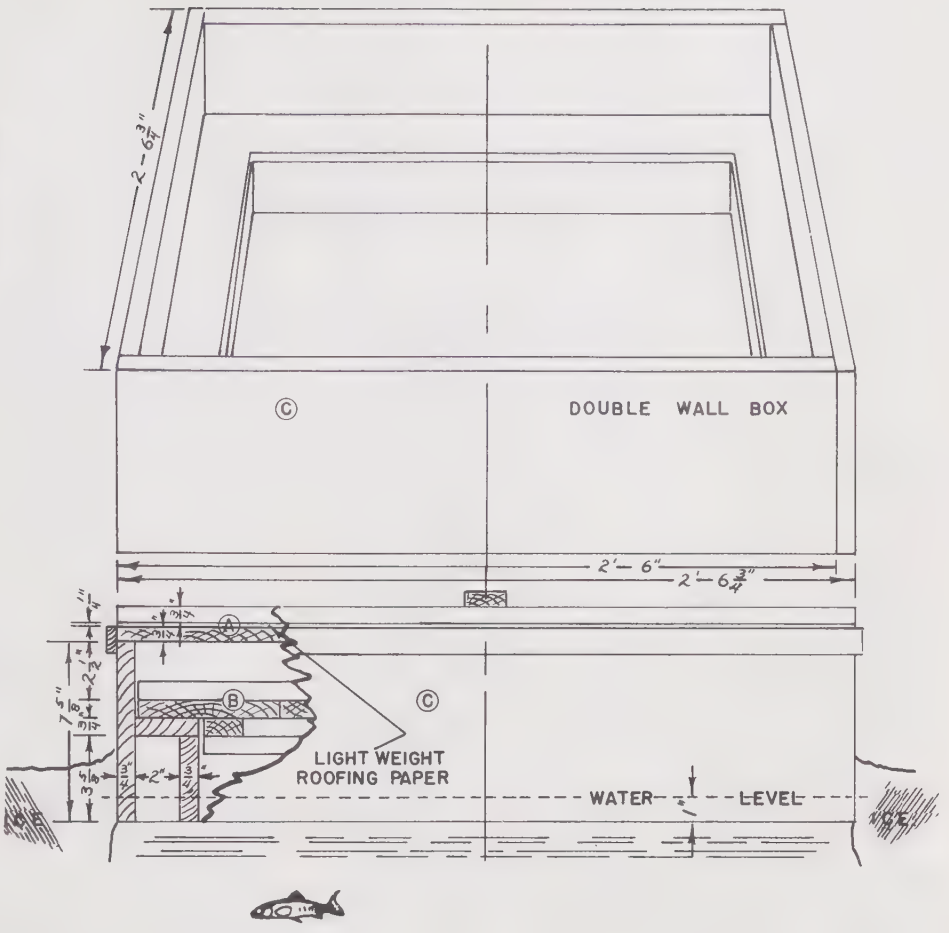


(Drawings by
Glyn A. Roberts)



installed 1 inch below the water level.

The thermos box, as we call it, has proved effective in preventing the formation of ice during temperatures of 30° below zero. So far, this box has been used only in moving water. It would be interesting to test the box in lakes and ponds that do not have circulation.



SHELL "ENDREX" USED AS A FISH TOXICANT

The extensive research on piscicides indicates that there is an urgent need for a cheap but effective fish toxicant for general use, quite apart from the need for specific toxicants.

For some time now we have been using very successfully an emulsifiable solution of Endrin that is known commercially as Shell "Endrex" to deal with predatory fishes in mining pools and fish ponds. Derris root, the plant from which rotenone is obtained, is grown in this country and is therefore readily available to us. We have found, however, that Shell "Endrex" is far cheaper and easier to use.

Applying Shell "Endrex" at a concentration of 0.008 p.p.m. Endrin, we have been able to harvest 10- to 15-pound carp from a mining pool 5 acres in extent and 10 to 20 feet in depth. Four hours after the application of Shell "Endrex," the fish came swimming near the surface, where they were easily taken from the pond by scoop-net. These carp were later sold in the local markets.

Further details of this work may be found in the paper entitled "The Use of Endrin as a Piscicide in Mining Pools and Fish Ponds in Malaya" by myself and A. B. O. Merican, presented during the Eighth Session of the Indo-Pacific Fisheries Council in December 1958.

---SOONG MIN KONG, Director of Fisheries Malaya, Federation of Malaya and Singapore, Penang.

REVIEW

RAINBOW TROUT IN MEXICO AND CALIFORNIA WITH NOTES ON THE CUTTHROAT SERIES

Paul R. Needham and Richard Gard

University of California Publications in Zoology, vol. 67, no. 1. 124 pp., 8 pl., 3 maps, 10 tables. University of California Press, Berkeley 4. 1959. $2.50.

The classification of the trouts and their allies has challenged taxonomists for more than a century. Although much has been learned about their biology and relationships, we still lack answers to fundamental questions bearing upon the systematics of this plastic group. Increased application of the experimental approach and of the study of internal characters (which are presumably less subject to rapid environmental modification) is needed to clarify the comparative roles of environment and heredity in molding the genus Salmo. It is hoped that the Needham and Gard work will stimulate such research.

Students of trout classification will be grateful to the authors of this ambitious study for their tedious work in analyzing certain morphometric and meristic data derived from 17 trout populations. The widely separated samples, all provisionally classified as rainbow trout (Salmo gairdneri), were taken from Eagle Lake, California, southward to the headwaters of Río del Presidio near Durango City, Mexico; all but 2 came from the Pacific slope, and 14 were collected in streams on the mainland of Mexico. Twelve counts and 28 measurements were made on 306 specimens (an average of 18 fish per locality) and are presented in the form of frequency graphs showing the variation in traits from north to south. The derived data are subjected to careful statistical treatment, and two-thirds of the text is devoted to an analysis of the characters and a detailed comparison of the samples. Twenty of the 28 measurements overlap and show no definitive pattern; only 6 of the 12 counts show differences of sufficient magnitude to distinguish any of the 17 populations.

There is a very useful history of trout collections from Mexico, a general discussion of rainbow trout taxonomy, some comments on the California golden trout (which is not assigned to S. gairdneri), a brief account of the effect of hatchery environment on morphometric characters, and a discussion of the cutthroat trouts (S. clarki). Instances are cited from marking experiments to show that some members of supposedly resident rainbow stocks have gone to the ocean to return as steelheads.[1/] The authors point out that the migratory urge may express itself repeatedly "wherever and whenever the genetic background and suitable environmental conditions" permit. A general discussion and summary are followed by literature citations (including unpublished studies) which bring the subject up to date.

[1/] See also the example of the development of a nonmigratory form of Pacific salmon near Vladivostok (U. S. Fish and Wildlife Service, Sport Fishery Abstracts, 3 (4): 200. Translation).

The most significant and interesting result of the field work in northwestern Mexico is the discovery of the Mexican golden trout, which is portrayed by a colored frontispiece that Dr. Needham assures me is a faithful rendition of its life appearance. This trout stands out from all of the other populations studied (and from any rainbow trout that I have seen) in its life colors; reduced number of vertebrae and branchiostegal rays; larger scales in the lateral line and above it; the slender, narrow, and short head; the short upper jaw and snout; bold spotting on the dorsal and caudal fins; and no or very little spotting below the lateral line or on the head. This fish is found in three separate but adjacent river systems and, with the exception of 7 specimens (of 90 that were analyzed), is consistent in its characters.

Although the Mexican golden trout is sufficiently well marked to warrant its nomenclatural recognition, the authors feel that its distinctive traits represent unstable features "that are easily and quickly modified by environmental changes." Even though the meristic and and morphometric characters chosen for study "were those that presumably would be most affected by the environment," I find it difficult to believe that all of the outstanding features of the Mexican golden trout are due to direct environmental effect. The golden trout is very similar in the three river systems, yet just to the south and to the north of its range are trout that look surprisingly like ordinary rainbows of coastal California.

This poses a critical question. Are the present stocks of trout in northwestern Mexico truly representative of the original fish? Concerted efforts were made during the field work to discover whether trout had been planted in this region, with negative results. However, some stocking could have been done privately, with no record. I am aware of one such planting in the Yaqui basin of northern Sonora, and at least one other seems to have occurred. On the east slope of the Sierra Madre Occidental, in the Rio Casas Grandes, there is a rainbow trout (plate 5b) very similar to the one in Black Canyon (in the headwaters of the Yaqui). Although the authors explain the existence of these "same phyletic stocks" by deriving the Casas Grandes form from that in the Yaqui via stream capture, the capture took place in the opposite direction. [2] Hence it is more than likely, as the authors themselves suggest (page 65), that the Casas Grandes rainbows were put there by man. There is also a suggestion, from the occurrence of a few individuals with exceptionally high scale counts (page 49 and figure 5), that the present Casas Grandes rainbow trout may be carrying some genes (from earlier hybridization and introgression) of a native cutthroat trout that gained access to the Casas Grandes from the Rio Grande. Although that species has been reported from the upper Yaqui, no specimens of S. clarki have been taken from this part of Mexico; however, Cope (in 1886) did report what evidently was a cutthroat from Pacific streams farther to the south; unfortunately, these fish have not been found.

Other suggestions that trout may have been introduced and that hybridization with native stocks may have taken place along the mainland of Mexico are: (1) The V-shaped clinal trend illustrated in many of the graphs, showing that the most distinctive populations (Mexican golden trout) occur in the middle of the area rather than at the extreme southern (or northern) limits; (2) in the Mexican golden trout complex, two samples are aberrant, more nearly resembling rainbows both to the north and south in certain features; (3) the sample from Rio Truchas, taken above a power dam and reservoir, has the general appearance of "typical rainbows." Stilwell's claim, [3] from secondhand information, that the engineers who built

[2] Miller, Robert R. 1959. Origin and affinities of the freshwater fish fauna of western North America. In: A symposium on zoogeography. Amer. Assoc. Adv. Sci., Publ. 51 (1958): 187-222.

[3] Stilwell, Hart. 1948. Fishing in Mexico. 296 pp. Alfred A. Knopf, New York.

the dam introduced the trout is strongly disputed by local testimony. (Stilwell also claimed that English immigrants stocked trout in northwestern Mexico in the 1880's; and, in 1955, Needham agreed.[4] Though trout are surely native in northwestern Mexico, we certainly have reason to believe that some of the present stocks are contaminated.

The authors contend (pages 3, 46) that even if hatchery trout had been stocked in these streams, it is unlikely that the native gene pools would have become contaminated. This conclusion may be challenged. When rainbow trout are introduced into streams containing only native cutthroat trout (S. clarki), a large part or even all of the population may show introgression of traits. Thus "rainbows" and "cutthroats" of Alberta clearly exhibit a number of features which demonstrate that mutual intermixing of genes has occurred,[5] and the two species have fused in Grebe Lake, Wyoming.[6] The native trout of the Gila River basin in New Mexico and Arizona has been reduced to the verge of extinction, in part through hybridization and introgression of characters, so that it is now often difficult to be certain of the purity of the original stock (personal observations).

The discussion of the cutthroat trout trout series contains valuable information on the natural history of this species, some of which is summarized from previously unpublished studies. An interesting series of photographs of the coastal form is shown in plate 8. Most, but not all, coastal cutthroats migrate to the ocean during some phase of their life cycle but spend relatively little time in salt water, primarily in estuaries. Differences in time of spawning and in preferred spawning site explain why hybrids between cutthroats and rainbows are almost unknown where the two species are naturally sympatric. The cutthroat trout of Independence Lake, California, is described as a unique surviving population of the native Lahontan cutthroat. The authors rightly bemoan the general failure of fishery biologists and conservationists to protect and develop such strains. Presented here are some unpublished data on artificially produced cutthroat-rainbow hybrids which confirm the belief that F_1 hybrids may be fertile, although to what degree is not shown. There appears to be no sound basis for suspicion that future studies will lead to the merging of S. clarki and S. gairdneri.

Needham and Gard have inaugurated an important and controversial study which reopens the problem of trout classification and suggests ways for a solution. The need for experimental work is stressed, and we are now afforded a unique opportunity to apply this approach to the long-isolated, distinctive Mexican golden trout. Additional field work is also called for, as half of the 14 samples from Mexico contain too few specimens (2 to 14) for thorough comparative treatment. In my critical perusal of the paper for this review, I have at times had the uneasy feeling that the derived data, rather than the fish populations, have too often been overstressed. The possibility that cutthroat trout still exist somewhere in the rugged mountains of northwestern Mexico merits careful investigation. Fresh material is needed for studies of comparative osteology, which may contribute importantly to a more sound basis for classification. Lastly, a thorough knowledge of the entire fish fauna of northwestern Mexico may help to reveal the probable course of evolution in the trouts.

ROBERT RUSH MILLER, Museum of Zoology, University of Michigan, Ann Arbor, Michigan.

[4] Needham, Paul R. 1955. Trail of the Mexican trout. Pacific Discovery, 8 (4): 18-24.

[5] Miller, Richard B. 1957. Have the genetic patterns of fishes been altered by introductions or by selective fishing? Jour. Fish. Res. Bd., Canada, 14 (6): 797-806.

[6] Kruse, Thomas E. 1959. Grayling of Grebe Lake, Yellowstone National Park, Wyoming. U. S. Fish and Wildlife Service, Fish. Bull. 149: 307-351.

TO CONTRIBUTORS

Contributions for publication in THE PROGRESSIVE FISH-CULTURIST are always welcome. All contributions should be directed to: The Editor, THE PROGRESSIVE FISH-CULTURIST, Bureau of Sport Fisheries and Wildlife, Washington 25, D. C.

Manuscripts should be typewritten, double-spaced, on one side of white bond paper. Illustrations should be in the form of sharp, glossy, black-and-white photographs or drawings prepared with waterproof black India ink on heavy white paper or tracing cloth.

A short abstract should be submitted with each manuscript. When an article has appeared in an issue of THE PROGRESSIVE FISH-CULTURIST, the publication data will be added to the abstract and this will then be forwarded to Biological Abstracts and Sport Fishery Abstracts.

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